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EVALUATION OF THE VESICATING PROPERTIES OF NEUTRALIZED CHEMICAL AGENT IDENTIFICATION SET (CAIS) COMPONENTS

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13. ABSTRACT *(Maximum 200 words)*
Vesication and skin irritation studies were conducted in hairless guinea pigs to ascertain the vesicant and skin irritation potential of chemically-neutralized Chemical Agent Identification Sets (CAIS). CAIS are training items that contain agent (HD, HN, or L) and were declared obsolete in 1971. Animals were dosed with "test article" [either neat HD, 10% agent/chloroform solutions or product solutions (wastestreams) and evaluated for skin-damaging effects (gross and light microscopic). Product solutions from the chemical neutralization of CAIS (either agent/chloroform or agent/charcoal) produced no microblisters. Dermal application of product solutions from the neutralization of neat HD resulted in microvesicle formation (vesication). All agent-dosed (either agent/chloroform solutions or HD) sites exhibited microblisters as well as other histopathologic lesions. Skin irritant effects were consistent with the skin-injurious activity (erythema and edema) associated with the neutralizing reagent [1,3-dichloro-5,5-dimethylhydantoin (DCDMH)].

14. SUBJECT TERMS

Acute toxicity (dermal)	Guinea pig (hairless)	HD
Microvesication	Wastestreams	HN
Histopathology	Rapid Response System (RRS)	L

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Test article administration - dermal	2/19/96	3/4/96	3/4/96
Test system preparation	2/19/96	3/4/96	3/4/96
Gas chromotography analysis	2/20/96	3/4/96	3/4/96
Sample collection	2/20/96	3/4/96	3/4/96
Histology processing	2/22/96	3/4/96	3/4/96
Test system preparation	6/26/96	7/1/96	7/1/96
Test article administration - dermal	6/26/96	7/1/96	7/1/96
Histology processing	6/27/96	7/1/96	7/1/96
Necropsy/tissue collection	6/27/96	7/1/96	7/1/96
Euthanasia	6/27/96	7/1/96	7/1/96
Histology processing	6/28/96	7/1/96	7/1/96
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Elisha Morrison 6/5/97

MREF Quality Assurance Unit, Date

GLP COMPLIANCE STATEMENT

The percutaneous dosing of hairless guinea pigs with wastestreams, neutralizing solution and known vesicants, and the gross and histopathologic evaluations of skin lesions in this study were performed by Battelle in compliance with the Environmental Protection Agency's (EPA) Good Laboratory Practice (GLP) Standards (40 CFR Part 792). Likewise, evaluation of the analytical method for HD, HN-1 and L in wastestreams and the determination of HD or HD, HN-1 and L concentrations, as appropriate, in wastestreams was accomplished at Battelle in compliance with EPA GLP Standards. Reports on findings from searches of the literature on HD, HN-1 and L degradation and degradation products and their vesicancy potential as well as analyses of wastestreams for degradation products and residual agent concentrations performed elsewhere than the MREF are excepted from this Good Laboratory Practices Compliance Statement. This study was conducted according to the study protocol, as amended, and Battelle's standard operating procedures. Deviations from the protocol or standard operating procedures are documented in Appendix A. The data presented accurately reflect the results of this study.

Carl T. Olson

Carl T. Olson, D.V.M., Ph.D.
Study Director

6/5/97
Date

QUALITY ASSURANCE

The analytical data supplied by the U. S. Army Edgewood Research, Development and Engineering Center (ERDEC) in support of this task were generated under the auspices of the Research and Technology Directorate Quality Assurance Program Plan. Accordingly, the data are supported by written methodology, sample identification records, and suitable instrument maintenance and calibration. The data and supporting records are retained by ERDEC.

Dennis W. Johnson

DENNIS W. JOHNSON
Quality Assurance Coordinator
Research and Technology Directorate

PREFACE

The work described in this report was authorized by the Project Manager Non-stockpile Chemical Materiel. This work was started in November 1995 and completed in August 1996.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," National Institute of Health Publication 85-23, 1985, as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, Commission of Life Sciences, National Research Council (Washington, DC). These investigations were also performed in accordance with the requirements of AR 70-18, "Laboratory Animals, Procurement, Transportation, Use, Care, and Public Affairs," and the Laboratory Animal Use and Review Committee (LAURC), U.S. Army Edgewood Research, Development and Engineering Center (ERDEC), which oversees the use of laboratory animals by reviewing for approval all ERDEC research protocols requiring laboratory animals. This project, assigned LAURC Protocol No. 21095000A302C, was approved on 5 Dec 1995.

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EVALUATION OF THE VESICATING PROPERTIES OF NEUTRALIZED CHEMICAL AGENT IDENTIFICATION SET (CAIS) COMPONENTS

1. Introduction

The U.S. Army Project Manager for Nonstockpile Chemical Materiel has a requirement to develop and field an on site system [Rapid Response System (RRS)][1] to demilitarize the chemical vesicating (blistering) agents sulfur mustard (HD), nitrogen mustard (HN), and Lewisite (L) contained in Chemical Agent Identification Sets (CAIS). Within the CAIS are glass ampules containing: HD (5 percent), HN (10 percent), or L (5 percent) in chloroform; HD, HN, or L in a charcoal matrix; and neat HD. The proposed RRS operation consists of removing ampules from CAIS and crushing them in a decontamination solution under engineering controls. After chemical neutralization of the agents, the wastestreams are to be turned over to a contractor for ultimate disposal by incineration. The intent of the neutralization process is to produce wastestreams that can be handled in a manner similar to that for industrial wastes. The wastestreams are to be regarded as hazardous material and dealt with in accordance with regulatory guidelines.

Previous research efforts related to the demilitarization/detoxification of CAIS[2] were conducted in the mid 1970s (Rescigno and Duggan, 1977; Rosenberg, 1977), and recent studies were conducted to ascertain the dermal toxicity characteristics of various RRS wastestreams (Olajos, *et al.* 1996). The current studies stemmed from the need to develop effective chemical neutralization processes which assure the reduction of agent vesicancy. This report provides an account of studies on the vesicancy potential of RRS wastestreams generated from the treatment of CAIS with chemical neutralization reagent.

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[1]RRS is a transportable system for identification, segregation, repackaging and/or treatment of CAIS.

[2]Chemical detoxification is defined as a process to convert chemical agents to products that do not exhibit the toxic properties of chemical warfare materiel (CWM). This process is also known as chemical neutralization as defined in Army Regulation 385-61.

2. Materials and Methods

2.1 Chemicals

2.1.1 Agents

Sulfur mustard [2,2'-dichlorodiethyl sulfide (HD), CAS #505-60-2] furnished from Medical Research and Evaluation Facility (MREF) stocks was used neat (undiluted) as a positive control article for vesication.[3] Lewisite [dichloro-2-chlorovinyl arsine (L)] CAS #541-25-3 was also furnished from MREF stock. U.S. Army Edgewood Research, Development and Engineering Center (ERDEC) provided a 20 percent solution of nitrogen mustard [bis (2-chloroethyl) ethylamine (HN-1), CAS #538-07-8] in chloroform. Ten percent solutions of HD, HN-1, and L in chloroform were prepared within the MREF laboratory and were used, along with neat HD, as control articles to demonstrate the ability of known vesicants to produce microvesicles and other histopathology when used to dose hairless guinea pigs percutaneously.

2.1.2 Chemical Agent Identification Sets (Synthesized)

Actual ampules from CAIS kits were not used; however "CAIS components" were prepared by ERDEC from agent stocks to contain 10 percent agent in chloroform (Chatfield, *et al.* 1995). Chemical Agent Standard Analytical Reference Material (CASARM) grade HD CAS# 505-60-2 (97.5 mole %), nitrogen mustard [bis (2-chloroethyl) ethylamine (HN-1)] CAS #538-07-8 (≥97% by weight), and CASARM grade lewisite [dichloro-2-chlorovinyl arsine (L)] CAS #541-25-3 (97.8 % by weight) from stocks maintained by the Operations Directorate, ERDEC were used in the preparation of synthesized CAIS. CASARM for HN-1 is not available.

2.1.3 Neutralized Chemical Agent Identification Sets (Wastestreams)

Wastestreams were provided by ERDEC, Aberdeen Proving Ground, MD. Wastestreams from the chemical neutralization of "CAIS components" prepared from agent stocks were tested for vesicancy potential. These wastestreams were prepared by ERDEC as follows:

- Wastestreams from the neutralization of neat HD with 1,3-dichloro-5,5-

[3]The chemical agents found in CAIS include sulfur mustard, nitrogen mustard, or lewisite. Sulfur mustard was used as representative vesicant for these blistering agents.

dimethylhydantoin (DCDMH) in $CHCl_3$/t-BuOH/3% H_2O. ("Blue" process)

- Wastestreams from the neutralization of 10% HD, HN, or L (agent in $CHCl_3$) with DCDMH in $CHCl_3$/t-BuOH/3% H_2O. ("Red" process)

- Wastestreams from neutralization of HD, HN, or L (agent on charcoal) with DCDMH in $CHCl_3$ (HD, HN samples) and with DCDMH in $CHCl_3$/t-BuOH/3% H_2O (L sample). ("Charcoal" process)

Two wastestreams ("archived"[4] and "fresh"[5]) were prepared for each process - "Blue", "Red", and "Charcoal" - and samples sent to the MREF for analysis of agent content and for vesicancy testing. The stability of the wastestreams under conditions of administration were not determined by MREF personnel. Test articles are "archived" and "fresh" "Blue", "Red", and "Charcoal" wastestreams.

2.1.4 Neutralization Solution

Neutralizing solution was prepared at the MREF to determine the effect on the skin of dosing this solution alone. For testing vesicating potential, a 0.555M 1,3-dichloro-5,5-dimethylhydantoin (FW 197.02) control article neutralizing solution was prepared by adding 10.9 g DCDMH to a 50:50 tertiary butanol:chloroform with 3 percent water solution in a 100-mL volumetric flask and adding sufficient volume of the butanol/chloroform/water solution to bring the volume to the 100-mL mark. DCDMH (CAS #118-52-5) was purchased from Aldrich Chemical Company (St. Louis, MO). Chloroform (CAS #67-66-3; GC/Spectro grade) was purchased from Burdick and Jackson (Muskegon, MI), and tertiary-butyl alcohol (CAS #75-65-0; ACS Reagent grade) from J.T. Baker (Phillipsburg, NJ). Distilled water was further purified using a Millipore (Bedford, MA) reverse osmosis system.

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[4]"Archived" "Blue" and "Red" wastestreams were initially analyzed at ERDEC Oct 95 and re-analyzed for agent residual at the MREF and tested for vesicancy. "Charcoal" wastestream initially analyzed at ERDEC Nov 95 was re-analyzed and tested for vesicancy at the MREF.

[5]"Fresh" wastestreams were prepared and initially analyzed at ERDEC and re-analyzed and tested for vesicancy at the MREF.

2.2 Process Chemistry (Chemical Neutralization Technology)

Process chemistry development was dictated by the requirement for both chemical neutralization and effective detoxification of the agent. Formulations of treatment reagent/solvent systems for the chemical neutralization of CAIS, as reported by ERDEC, are presented in Table 1. Using the neutralization processes, the chemical agents in CAIS may undergo oxidation/chlorination/substitution to yield a mixture of products/by-products, and residual agent may also be present in the wastestreams (Olajos, *et al.* 1996).

TABLE 1. OXIDIZER/SOLVENT SYSTEM STOICHIOMETRY UTILIZED IN THE MODIFIED "BLUE", "RED", AND "CHARCOAL" PROCESS CHEMISTRIES

- 1 volume of neat HD treated with 20 volumes of 0.555M 1,3-dichloro-5,5-dimethylhydantoin (DCDMH) in $CHCl_3$/t-butanol (50/50) with 3% water by volume. ("Blue" Process)
- 1 volume of each 10% HD in $CHCl_3$, 10% HN in $CHCl_3$, and 10% L in $CHCl_3$ treated with 4 volumes of 0.555M 1,3-dichloro-5,5-dimethylhydantoin (DCDMH) in 50/50 $CHCl_3$/t-butanol with 3% water by volume. ("Red" Process)
- 45% by weight HD and HN-1 on charcoal treated with excess 1,3-dichloro-5,5-dimethylhydantoin in $CHCl_3$ combined with 43% by weight L with excess 1,3-dichloro-5,5-dimethylhydantoin in $CHCl_3$/t-butanol (50/50) with 3% water by volume. ("Charcoal" Process)

2.3 Analytical Methodologies

2.3.1 GC-MS Spectroscopy

Chemically-treated (neutralized) CAIS were analyzed for agent residue levels using full scanning gc-ms spectroscopy. GC-MS spectroscopy was conducted at ERDEC on all wastestreams provided to the MREF, and confirmatory gc-ms analysis was also performed at the MREF prior to conducting the bioassays.

Instrumentation used in the ERDEC analysis of "archived" wastestreams (non-quenched samples) was a Hewlett-Packard 5989B MS engine with Chemstation Data System. Analysis conducted at both ERDEC and the MREF on "fresh" wastestreams, using quenching and derivatization techniques (Dr. Samuel Lucas of Battelle), utilized a Hewlett-Packard Model

5970B Mass Selective Detector (MSD) with an HP 5890A GC and HP 61034 CMS. For procedural details, the reader is referred to Rosso (1995), as provided in ERDEC-TR-372 (Olajos, *et al.* 1996), and Appendix B of this report. Quantitation was based on internal standardization (internal standard = 1,2,4,5-tetrachlorobenzene). Calibration standards were as follows: HD (purity 97.5 %), HN-1 (purity 96.5 %) and L (purity 97.8 %).

Product identification of the CAIS wastestreams was accomplished using gc/ms spectroscopy (EI and CI modes). These studies were performed by ERDEC chemists per procedures outlined in ERDEC Analytical Chemistry Method (Rosso *et al.* 1995; See also Appendix C). The predominant instrument for component identification via the chemical ionization (CI) mode was a Finnigan 5100 gc/ms. The mass spectrometer was operated in the CI mode with methane as the CI reagent gas at a source pressure of 0.5 Torr. Scan time was one sec per scan, and the scan range was 60 to 450 amu. Procedural details have been reported (Olajos, *et al.* 1996).

2.3.2 NMR Spectroscopy

Nuclear magnetic resonance (nmr) spectroscopy analyses of "fresh" wastestreams were conducted at ERDEC as an adjunct to gc-ms analyses. These analyses were performed using a Varian Fourier Transform (FT) nmr spectrometer operated at 200 MHZ for ^{1}H observation and at 50 MHZ for ^{13}C observation. Quantitative data were obtained by digital integration of peak areas.

2.4 Animals and Housing

A total of 35 male (approximately 200-350 g and 3 to 4 weeks of age upon receipt), euthymic hairless guinea pigs (Crl:IAF (HA)-hr BR), procured from Charles River Laboratories (Wilmington, MA; animals supplied from Portage, MI facility), were used in this study. In this species, the percutaneous application of a vesicating agent such as HD produces the formation of microblisters or microvesicles, a separation of epidermis from dermis of two or more cell widths due to destruction of basal cells (Marlow, *et al.* 1990, Mershon, *et al.* 1990, Braue, *et al.* 1992, and Yourick, *et al.* 1992). This is analogous to the changes seen in man (Papirmeister, *et al.* 1984).

Animals were quarantined and screened for general condition and health status, and were maintained in a program accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC) International. Ear tags were applied to maintain positive identification, and animals were maintained between approximately 64 and 79 degrees F and 40 to 70 percent relative humidity with a 12-hr diurnal light cycle. Food and water were provided *ad libitum* and animals were housed individually in polycarbonate cages prior to exposure to "test article". Following treatment, animals were housed individually within a chemical fume hood during the 24-hr post-exposure period. Following recovery from anesthesia, animals were given food and water.

2.5 Experimental Design

The task was designed to be accomplished in three phases. The first phase was analytical chemistry. This included sample analysis, which was preceded by an evaluation of the limit of detection, the limit of quantitation, the linearity of response, and the precision, accuracy, and specificity of the methodology. Following evaluation of the analytical method, analyses of wastestreams for residual HD ("Blue") or HD, HN-1, and L ("Red" and "Charcoal") were to be accomplished.

The objective of phase II was to assess the biologic effects of dosing volume and exposure time for CAIS components. Two sets of experiments were conducted in Phase II. In the first set, each animal was dosed percutaneously with various volumes of each 10% agent in chloroform solution and with neat HD to determine a volume and a duration of exposure for each agent preparation that resulted in consistent production of microvesication. Once a dosing volume and a duration of exposure for each agent solution were selected, the second set of experiments was conducted to verify consistent microvesication following dosing of agent solutions and to assess the extent of skin pathology following dosing of the neutralization solution alone. Dosing volume of neutralization solution was based upon the approximate volume used to neutralize that volume of agent/agent solution that was determined to consistently create microvesication. Up to eight sites on each guinea pig were dosed and at approximately 24 hr after dosing, using a modification of the Draize method (Draize, *et al.* 1944), the extent of erythema and edema was graded and lesion size was measured at each site. The animals were then sacrificed and skin samples taken from dosed sites and prepared for histopathologic evaluation. Histopathologic lesions (microblisters, epidermal necrosis, follicular necrosis, dermal necrosis, vascular necrosis, hemorrhage, and pustular epidermitis) were graded by a veterinary pathologist. Eleven animals were dermally dosed with 1 μL neat HD and with 10 percent agent (HD, HN or L) in chloroform solutions with dosing volumes ranging from 5 to 50 μL and exposure times of 1 or 2 hours. Five of these 11 guinea pigs were treated with the DCDMH oxidant/solvent neutralizing solution.

Phase III was to demonstrate that the neutralization process substantially reduced the vesicating properties of wastestreams. Each animal was dosed percutaneously with agents using parameters established in Phase II and with volumes of wastestreams (25 μL) selected such that the volume of agent in the "Blue" or "Red" treated wastestream that potentially could not be neutralized was approximately equivalent to that agent quantity which when dosed on animal backs created microvesication. For consistency, the same 25 μL of "Charcoal" wastestream was dosed. The exposed skin was examined 24 hours after dosing and then harvested for histopathologic examination.

The experimental protocol is attached as Appendix A. A synopsis of the experimental design is given in Table 2.

2.6 Animal Preparation and Dosing

Initially using 6 mg xylazine hydrochloride and 35 mg ketamine hydrochloride per kg body weight given intramuscularly and increasing this to 13 mg xylazine and 87 mg ketamine/kg following the first day of dosing, anesthetized guinea pigs were dosed percutaneously on both sides of the dorsal midline with "test articles" (six to eight exposure sites/animal) - see Fig. 1. Table 2 presents a synopsis of treatments, application volumes, and exposure durations. Techniques for dosing are described in Battelle SOP MREF II-009 (Appendix A). Following the requisite exposure time, the exposed skin was decontaminated with 0.5 percent sodium hypochlorite solution (non-irritating concentration). Approximately 24 hours after dosing, the animals were again anesthetized, sites evaluated for erythema and edema and lesion size, and animals then sacrificed with an inhalation anesthetic (halothane) overdose. Following euthanasia,

TABLE 2. SYNOPSIS OF PHASE II AND PHASE III TESTING PROCEDURES

Phase II. A total of 11 hairless guinea pigs (HGPs) were used in this phase with seven or eight sites dosed on each animal. All animals were examined 24 hr following exposure and sites evaluated for erythema and edema. Following this evaluation, animals were euthanatized and dosed skin harvested for histopathology studies.

Test Article	Volume Dosed (μL)	Duration of Exposure (hr)	Total No. HGPs/Total No. Sites Dosed
10% Agent/Chloroform			
HD, HN, or L	5	2	2/2 for each agent solution
	10	2	4/4 for each agent solution
	50	2	2/2 for each agent solution
	5	1	7/7 for each agent solution
	10	1	2/2 for each agent solution
Neat HD	1	2	4/4
	1	1	7/7
Oxidant/Solvent	20	1	5/20

Phase III. A total of 24 HGPs were used, dosing six to eight sites per animal with a 1 hr duration of exposure. All animals were examined 24 hr following exposure and sites evaluated for erythema and edema. Following this evaluation, animals were euthanatized and dosed skin harvested for histopathology studies. Two separate "wastestreams" from each process were provided for testing. The initially provided wastestreams were labeled "archived" and the second set "fresh".

Test Article	Volume Dosed (μL)	Total No. HGPs/Total No. Sites Dosed
10% Agent/Chloroform		
HD, HN, or L	5	20/20 for each agent solution
HD, HN, or L	10	4/4 for each agent solution
Neat HD	1	16/16
Wastestream		
"Archived" "Blue" (11/28/95)[a]	25	8/8
"Archived" "Red" (11/28/95)	25	8/8
"Archived" "Charcoal" (1/25/96)	25	8/8
"Archived" "Blue" (11/28/95)	10	4/4
"Archived" "Red" (11/28/95)	10	4/4
"Archived" "Charcoal" (1/25/96)	10	4/4
"Fresh" "Blue" (6/19/96)	25	8/16
"Fresh" "Red" (6/19/96)	25	8/16
"Fresh" "Charcoal" (8/29/96)	25	4/12

[a] Date is when wastestream was received at the MREF.

skin samples were collected and processed for histopathology.

2.7 Histopathologic Analysis

Following euthanasia, skin from the dosed sites was taken and placed in buffered formalin. After fixation, embedding, and sectioning, skin samples were stained with hematoxylin and eosin and evaluated for histopathology. Histopathologic lesions (microblisters, epidermal necrosis, follicular necrosis, dermal necrosis, vascular necrosis, hemorrhage, and pustular epidermitis) were graded on a scale of 0-4, where 0 = normal, 1 = minimal, 2 = intermediate, 3 = moderate, and 4 = severe. Definitions for scoring of histopathology and the criteria for grading severity of lesions are summarized in Table 3. The grading of microblister formation is highlighted in Table 4.

FIGURE 1. GUINEA PIG SKIN EXPOSURE SITES

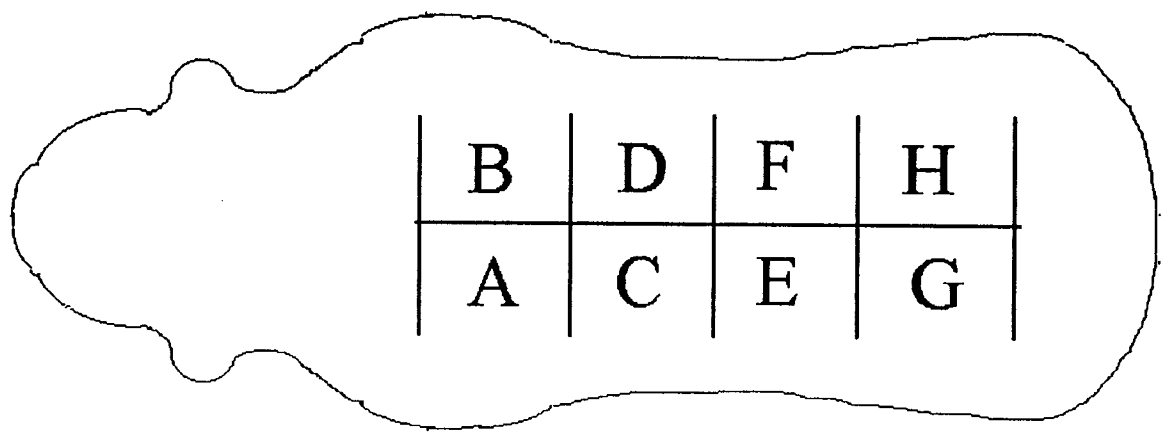

TABLE 3. DEFINITIONS USED IN HISTOPATHOLOGIC EVALUATIONS AND AN EXPLANATION OF THE GRADING OF LESION SEVERITY

Microblister	Loss of epidermal basal cell attachment to the underlying basement membrane of at least two adjacent cells. The loss of attachment creates a space which may appear empty, full of proteinaceous fluid, or filled with neutrophils. One or a few isolated small areas of detachment is graded 1, minimal. Many such areas of detachment, or several larger (10 or more contiguous cells) areas of detachment is graded 2, intermediate. When half or more of the epidermis in the tissue section is detached from the dermis, it is graded 3, moderate. Such lesions typically have a much larger space between the basal cells and the dermis. When nearly all of the epidermis is separated from the dermis, it is graded 4, severe. In such situations, there are usually focal, point attachments, so the entire epidermis is not lifted along the full width of the section.
Epidermal necrosis	The epidermal cells exhibit cytoplasmic eosinophilia, nuclear loss or pyknosis, and are generally shrunken. If only individual cells are affected, it is graded 1 (these are generally isolated basal cells). If small areas are affected, with normal areas in close proximity, it is graded 2. If the epidermis exhibits cell death in a full-thickness (all layers of epidermis) pattern, and affects half or more of the skin section, it is graded 3. If the epidermis is virtually entirely necrotic, it is graded 4. Severe ulcers assume that the epidermis is necrotic.
Follicular necrosis	If isolated epithelial cells of the hair follicles exhibit eosinophilia or pyknosis, it is graded 1. If clusters of adjacent cells within follicles are dead, it is graded 2. If cells of half or more of a particular hair follicle are dead, it is graded 3. Grade 4 lesions have complete necrosis of the follicular epithelium underlying much of the epidermal lesion area. This indicates that the agent has penetrated deeply.
Dermal necrosis	Loss of collagen fiber integrity, evidenced by pale eosinophilic staining and homogeneous appearance, indicates necrosis of dermal fibers. With only isolated areas, it is graded 1. Multiple areas are graded 2. Necrosis of most of the superficial dermal collagen in the lesion area is graded 3. A grade 4 lesion requires deep (to the base of the associated adnexa) dermal necrosis.
Vascular necrosis	Loss of integrity of a medium to large blood vessel is vascular necrosis. Grading depends upon the number of vessels affected and the severity. Partial necrosis of one vessel is graded 1 to 2. Complete necrosis of a vessel is graded 3; multiple such lesions are graded 4.
Hemorrhage	Extravasated erythrocytes is hemorrhage. A few isolated foci is graded 1. Multiple, common foci is graded 2. Large pools of blood is graded 3. A grade 4 lesion requires a massive area of blood pooling and the displacement of large areas of dermal collagen.
Pustular epidermitis	Collections of neutrophils in the epidermis proper is graded by extent; one or two small foci is graded 1; three or more small foci is graded 2; one or more large foci is graded 3; a grade 4 lesion would indicate massive infiltration of the entire epidermis by neutrophils.

TABLE 4. DEFINITION OF DEGREES OF SEVERITY USED FOR HISTOPATHOLOGIC EVALUATION OF VESICATION (MICROBLISTER FORMATION[a])

Lesion Characteristic	Degree of Severity
➡ No lesion (unaffected)	0 (normal)
➡ One or a few isolated areas of detachment	1 (minimal)
➡ Many small areas of detachment or several larger areas of detachment	2 (intermediate)
➡ >50% of the epidermis in tissue section is detached from the dermis (much larger space between basal cells and dermis)	3 (moderate)
➡ Nearly all the epidermis is separated from the dermis	4 (severe)

[a] Microblister: loss of epidermal basal cell attachment to underlying basement membrane of at least two adjacent cells. Loss of attachment creates a space.

2.8 Data Analysis

For chemistry data generated in Phase I, means and standard deviations of responses of each control standard were determined to calculate both the inter- and intra- variability of the analytical method. Calibration performance characteristics for each analyte, such as slope and standard error of the slope, R^2 (measure of fit about the regression line), method detection limits, and quantitation limits were calculated.

For Phase III data (vesicating assessment of wastestreams), statistical hypothesis tests were conducted at the 5 percent significance level to determine whether or not the neutralization process reduced the vesicating property of agents contained in CAIS. For each CAIS sample, the incidence of microblisters at sites treated with CAIS agent(s) were compared to those of contralateral sites treated with the wastestream. Although incidence of microblisters was the primary endpoint for evaluating the efficacy of each neutralization process, analyses were also conducted on other indices of skin injury (gross and microscopic). To accommodate the intra-animal correlation of multiple measurements made on the same animal, McNemar's test was used to analyze quantal data (Agresti, 1990). Analysis of variance (ANOVA) models, that include random effects for animal, were fitted to continuous data. If data were not approximately normal, ANOVA were conducted on transformed data, or nonparametric or categorical methods of analysis were performed.

3. Results

3.1 Chemistry

Nitrogen mustard, sulfur mustard, and lewisite are components of CAIS that were chemically neutralized ("detoxified") on reaction with treatment reagent (1,3-dichloro-5, 5-dimethylhydantoin). The selection of a particular process chemistry (designated as "Blue", "Red", or "Charcoal" process) was dependent on whether the agent was neat material (HD), in solution (agent in chloroform), or adsorbed on charcoal.

The DCDMH-mediated neutralization of sulfur mustard resulted in HD concentrations below 50 ppm in "Blue" process wastestream (product solution). The DCDMH reaction resulted in the conversion of sulfur mustard to HD sulfoxide degradation products. Secondary reactions (i.e., elimination/substitution) also occurred which produced chlorinated and vinyl sulfoxides (Olajos, et. al., 1996).

The neutralization reaction between oxidant (DCDMH) and CAIS containing agent (HD, HN or L in chloroform - "Red" Process) resulted in fairly complex product solutions containing various products/by-products and residual amounts of un-reacted agent. Agent levels in "Red" process wastestreams were below 50 ppm for each agent (HD, HN or L) (Ibid).

The process chemistry for the neutralization of CAIS components containing agent (HD, HN or L) on charcoal ("Charcoal" process), resulted in the formation of complex product solutions. Agent residue levels were below 50 ppm for HD and HN and below 85 ppm for L (Ibid).

The wastestreams were complex mixtures which pushed the analytical methodologies to the limits of sensitivity, mixture analysis capability, and structural elucidation. "Archived" and "fresh" wastestreams produced from the neutralization reactions were analyzed by gc-ms for agent residual both at ERDEC and at the MREF. The Method Quantitation Limit (MQL), the concentration level that can be quantitatively reproduced, varied with agent and the matrix analyzed. The analytical capability of the gc-ms method for HD and HN was improved via the utilization of quenched samples (see Table 5). The MQL for all agents in non-quenched samples was 50 μg/mL (50 ppm) as reported by Rosso (1995) and cited by Olajos, *et al.* (1996). For the quenched samples, the MQL was 15 μg/mL (15 ppm) for HD or HN and 85 μg/mL (85 ppm) for

L. Derivatization of lewisite as an enhancement technique in its analysis did not lower the MQL to the desired level (<50 ppm); however, the derivatization prior to quenching did provide reduced variance in the data. See Appendix B for evaluation of the analytical technique and analyses of wastestreams performed at Battelle. It has been proposed that the MQL for L may be lower than 85 µg/mL (Lucas, 1997).

The "archived" wastestreams were additionally analyzed for product/by-product composition at ERDEC (See Table 6 and Appendix C for analytical chemistry performed at ERDEC). HD sulfoxide and other degradation products resultant from secondary reactions (e.g., elimination, substitution) were detected in wastestream samples. HD sulfone and/or its vinyl containing derivatives, which are known vesicants, were not detected in the "Blue" process wastestream. Product analysis did not reveal HD sulfone or vinyl/divinyl analogs in the product solution obtained from the chemical neutralization of the "Red" wastestream. Product characterization of the "Charcoal" wastestream did not reveal HD sulfone; however, multichlorinated vinyl containing derivatives (non-vesicant) were present in the product

TABLE 5. DETECTION AND QUANTITATION LIMITS FOR GC/MS ANALYSES

Analyte	Process Wastestreams					
	"Blue"		"Red"		"Charcoal"	
	MDL[a]	MQL[b]	MDL	MQL	MDL	MQL
HD[c]		50 ppm		50 ppm		50 ppm
HD[d]	3 ppm	10 ppm	2 ppm	5 ppm	4 ppm	14 ppm
HN-1[c]	(*)[f]	(*)[f]		50 ppm		50 ppm
HN-1[d]	(*)[f]	(*)[f]	2 ppm	7 ppm	2 ppm	6 ppm
L[c]	(*)[f]	(*)[f]		50 ppm		50 ppm
L[d,e]	(*)[f]	(*)[f]	14 ppm	46 ppm	25 ppm	85 ppm

[a]MDL = Method Detection Limit
[b]MQL = Method Quantitation Limit
[c]non-quenched sample (Analysis conducted at ERDEC)
[d]quenched sample (Analysis conducted at Battelle's MREF)
[e]derivatized lewisite (L-der)
[f]"Blue" process wastestream contains no HN or L but only neat HD.

solution. Lack of detection of HD sulfone in these wastestreams does not necessarily indicate the absence of this moiety since detection interferences and/or masking by another analyte may have prevented HD sulfone detection.

Nuclear magnetic resonance (nmr) spectroscopy of "fresh" wastestreams conducted at ERDEC did not detect HD in the "Blue" process wastestream and did not detect any HD, HN, or L in the "Red" or "Charcoal" wastestreams. Numerous peaks were evident which were consistent with agent products/by-products (e.g., $ClCH_2CHClS(0)CH_2CH_2Cl$ from HD degradation; $ClNHCH_2CH_2Cl$ from HN degradation; and $ClCH = CHAs(OH)_2$ from L degradation). Many unidentified product peaks were also detected during nmr analyses.

TABLE 6. COMPARISON OF AGENT RESIDUE LEVELS, MAJOR PRODUCTS/BY-PRODUCTS, AND UNKNOWNS IN "ARCHIVED" WASTESTREAMS GENERATED FROM THE CHEMICAL NEUTRALIZATION OF CAIS[a]

	Wastestreams		
Component	"Blue" Process (area %/ ppm)[b,c]	"Red" Process (area %/ ppm)[b,c]	"Charcoal" Process (area %/ ppm)[b,c]
HD	50 ppm	50 ppm	50 ppm
HN	(*)[d]	50 ppm	50 ppm
L	(*)[d]	50 ppm	50 ppm
HD sulfoxide	(-)[e]	(-)[e]	(-)[e]
HD sulfone	(-)[e]	(-)[e]	(-)[e]
sulfones	(-)[e]	(-)[e]	(6.7%)
sulfoxides (multi-chlorinated)[f]	(23.9%)	(-)[e]	(1.0%)
vinyl derivatives	(0.4%)	(-)[e]	(-)[e]
chlorinated alkanes	(0.5%)	(5.3%)	(17.5%)
chlorinated alkenes	(1.1%)	(2.4%)	(40.8%)
other	(25.4%)	(28.4%)	(28.2%)
unknowns	(-)[e]	(1.8%)	(4.6%)
solvents ($CHCl_3$: t-BuOH)	(48.5%)	(60.4%)	(-)
Totals	**(99.8%)**	**(98.3%)**	**(98.8%)**

a All data derived from gc-ms analysis of sample wastestreams was conducted at ERDEC.
b Values based on analysis of non-quenched/non-derivatized samples via gc-ms (CI mode for components analysis; EI mode for agent residual).
c Area % calculated from the Total Ion Chromatogram (TIC) of the mass spectrometer. The area % is semi-quantitative; the intent is to show the area under the peak in comparison to other peaks in the chromatogram.
d (*) "Blue" process wastestream contains no HN or L since the CAIS contains only HD.
e (-) Denotes not detected.
f (e.g., tri, tetra-chloro derivatives of HD sulfoxide).

3.2 Dermal Effects

3.2.1 Gross Pathologic Findings

Phase II. All skin exposures to HD and agent/chloroform solutions containing 10 percent HD, HN or L resulted in gross skin lesions consisting of well-defined areas of edema and erythema of moderate to severe intensity. In some instances, large areas of ulceration with complete loss of the covering epidermis was evident. The skin irritant effects of HN and L were comparable to that produced by HD (refer to Table 7 and Appendix D). The skin-injurant effect of oxidant/solvent solution was minimal gross lesions (refer to Table 7 and Appendix D).

Phase III. The cutaneous injury (non-vesicant) effects after 1 hour exposure to HD, agent/$CHCl_3$ solutions, or CAIS wastestreams ("archived" and "fresh") were evaluated and are summarized in Table 8. Individual gross pathology data are presented in Appendix D. All agent-dosed sites demonstrated gross lesions. Skin lesions were assumed to be elliptical in shape, and lesion area was computed using the formula lesion area = length x width x $\pi/4$. Wastestream-induced dermal injury resulted in mild to moderate degrees of erythema and edema. Because a "fresh" "Charcoal" wastestream was not available for dosing with "fresh" "Red" and "Blue" wastestreams in June, results following dosing of the "fresh" "Charcoal" wastestream in August were combined with results of "archived" "Charcoal" wastestream dosing in March for statistical analyses. Results of these analyses are tabulated in Table 9.

TABLE 7. PHASE II - SKIN REACTION (ERYTHEMA AND EDEMA) FOLLOWING EXPOSURE TO HD, AGENT/$CHCL_3$ SOLUTIONS, AND OXIDANT/SOLVENT SOLUTION

Experiment Date/ Animal ID	Test Article	Dose Volume (µL)	Time to Decontamination (hr)	No. of Animals Tested	Erythema Score, Mean	Edema Score, Mean
02/19/96 (301, 305)	10% L/$CHCl_3$	10	2	2	3.0	3.0
	10% L/$CHCl_3$	50	2	2	3.0	3.0
	10% HN/$CHCl_3$	10	2	2	2.0	2.0
	10% HN/$CHCl_3$	50	2	2	2.0	2.0
	10% HD/$CHCl_3$	10	2	2	2.0	2.0
	10% HD/$CHCl_3$	50	2	2	2.5	2.0
	Neat HD	1	2	2	2.0	2.0
02/21/96 (306, 309)	10% L/$CHCl_3$	5	2	2	2.5	3.0
	10% L/$CHCl_3$	10	2	2	2.5	3.0
	10% HN/$CHCl_3$	5	2	2	2.0	2.0
	10% HN/$CHCl_3$	10	2	2	2.0	2.5
	10% HD/$CHCl_3$	5	2	2	3.0	3.0
	10% HD/$CHCl_3$	10	2	2	2.0	2.0
	Neat HD	1	2	2	2.0	2.5
02/27/96 (312, 316)	10% L/$CHCl_3$	5	1	2	3.0	3.0
	10% L/$CHCl_3$	10	1	2	3.0	3.0
	10% HN/$CHCl_3$	5	1	2	2.0	2.5
	10% HN/$CHCl_3$	10	1	2	2.0	2.0
	10% HD/$CHCl_3$	5	1	2	3.0	2.0
	10% HD/$CHCl_3$	10	1	2	2.5	2.0
	Neat HD	1	1	2	3.0	2.5
03/05/96 (311, 313, 315, 317, 324)	10% L/$CHCl_3$	5	1	5	3.0	2.8
	10% HN/$CHCl_3$	5	1	5	1.8	2.0
	10% HD/$CHCl_3$	5	1	5	2.4	2.4
	Neutralizing Solution	20	1	5	0.0	1.0
	Neat HD	1	1	5	2.4	2.6

TABLE 8. PHASE III. SKIN REACTION (ERYTHEMA AND EDEMA) FOLLOWING EXPOSURE TO HD, AGENT/$CHCl_3$ SOLUTION OR CAIS WASTESTREAMS

Date, Source of Wastestream	Test Article	Dose Volume (µL)	No. of Animals Tested	Erythema Score		Edema Score		Lesion Area (mm^2)	
				Mean	S.D.	Mean	S.D.	Mean	S.D.
03/13/96, 03/21/96 "Archived" Wastestreams	10% L/$CHCl_3$	5	8	2.9	0.3	3.0	0.0	95.4	22.2
	10% HN/$CHCl_3$	5	8	2.0	0.9	2.0	0.5	60.1	13.6
	10% HD/$CHCl_3$	5	8	2.6	0.7	2.1	0.8	107.9	35.8
	"Red" Wastestream	25	8	1.1 [a,b,c]	0.3	1.8 [a]	0.5	237.4 [d,e,f]	71.3
	"Blue" Wastestream	25	8	1.9 [a,c]	0.8	1.6 [a,c]	0.5	236.5 [d,e,f]	72.6
	"Charcoal" Wastestream	25	8	0.4 [a,b,c]	0.2	0.4 [a,b,c]	0.2	132.9 [e]	95.9
	Neat HD	1	8	2.8	0.5	2.9	0.3	180.2	53.1
06/20/96, 06/26/96 "Fresh" Wastestreams	10% L/$CHCl_3$	5	8	3.0	0.0	3.0	0.0	156.0	67.1
	10% HN/$CHCl_3$	5	8	1.9	0.3	2.1	0.3	82.0	30.8
	10% HD/$CHCl_3$	5	8	2.4	0.5	2.3	0.5	94.9	18.4
	"Red" Wastestream	25	8	0.3 [a,b,c]	0.3	0.3 [a,b,c]	0.3	46.2 [a]	52.3
	"Blue" Wastestream	25	8	1.8 [a,c]	0.5	1.6 [a,b,c]	0.5	220.6 [d,e,f]	42.0
	Neat HD	1	8	2.5	0.5	2.4	0.5	126.8	32.6
08/13/96 "Archived" Wastestreams	10% L/$CHCl_3$	10	4	3.0	0.0	2.8	0.5	212.6	35.6
	10% HN/$CHCl_3$	10	4	2.5	0.6	2.3	0.5	155.1	21.4
	10% HD/$CHCl_3$	10	4	2.3	1.0	2.3	0.5	178.7	34.9
	"Red" Wastestream	10	4	1.1 [a,b,c]	0.6	0.8 [a,b,c]	1.0	121.1 [a,c]	41.8
	"Blue" Wastestream	10	4	1.3 [a,b,c]	0.5	1.0 [a,b,c]	0.0	142.4 [a]	42.3
	"Charcoal" Wastestream	10	4	0.4 [a,b,c]	0.2	0.0 [a,b,c]	0.0	69.3 [a,b,c]	23.0
08/29/96 "Fresh" Wastestream	10% L/$CHCl_3$	5	4	3.0	0.0	2.8	0.5	113.9	33.1
	10% HN/$CHCl_3$	5	4	1.5	0.6	2.0	0.8	91.3	19.6
	10% HD/$CHCl_3$	5	4	3.0	0.0	3.0	0.00	89.5	26.2
	"Charcoal" Wastestream	25	4	0.0 [a,b,c]	0.0	0.0 [a,b,c]	0.0	0.0 [a,b,c]	0.0

Note: All times to decontamination were 1 hr.

a Mean is significantly less than that observed on sites treated with L.
b Mean is significantly less than that observed on sites treated with HN.
c Mean is significantly less than that observed on sites treated with HD.
d Mean is significantly greater than that observed on sites treated with L.
e Mean is significantly greater than that observed on sites treated with HN.
f Mean is significantly greater than that observed on sites treated with HD.

TABLE 9. PHASE III. SUMMARY STATISTICS FOR ERYTHEMA, EDEMA, AND LESION AREA AFTER DOSING OF "CHARCOAL" WASTESTREAMS[a]

Agent/ Compound	Dose Volume (μL)	No. of Animals Tested	Erythema Score		Edema Score		Lesion Area (mm^2)	
			Mean	S.D.	Mean	S.D.	Mean	S.D.
L (10% solution)	5	12	2.9	0.3	2.9	0.3	101.6	26.4
HN (10% solution)	5	12	1.8	0.8	2.0	0.6	70.5	21.4
HD (10% solution)	5	12	2.8	0.6	2.4	0.8	101.8	32.9
"Charcoal" Wastestream	25	12	0.3[b,c,d]	0.3	0.3[b,c,d]	0.3	88.6	100.7

Note: All times to decontamination were 1 hr.

a Pooled data from the "Charcoal" wastestream received 1/25/96 and dosed on 3/13 and 3/21/96 and wastestream received 8/29/96 and dosed the same day. Volume of "Charcoal" wastestream dosed was 25 μL at each site.

b Mean is significantly less than that observed on sites treated with L.

c Mean is significantly less than that observed on sites treated with HN.

d Mean is significantly less than that observed on sites treated with HD.

3.2.2 Histopathologic Findings

Phase II. Two hour dermal exposures of animals to neat HD (1 μL) and to various doses (5 - 50 μL) of agent/chloroform solutions containing 10 percent HD, HN or L resulted in microblister formation of moderate to severe intensity - refer to Table 10. Incidence of histopathologic changes are summarized in Table 11. In some animals, large areas of ulceration with loss of epidermis prevented the occurrence of microblisters. Individual animal histopathology data are presented in Appendix E. Based on the outcome of the two-hour exposure studies, other guinea pigs were dosed with 5 and 10 μL volumes of 10 percent agent in chloroform solutions and with neat HD (1 μL) at an exposure duration of one hour. Microblister formation was evident at all sites, unless occurrence was precluded by development of an ulcer, and ranged in severity from moderate to severe. The application of 5 μL of 10 percent agent/chloroform solution resulted in microblisters of at least intermediate severity. Refer to Table 10 for incidence/response summary and Appendix E for individual histopathologic findings. The oxidant/solvent system was also evaluated for skin effects. Animals treated with oxidant/solvent solution did not manifest dermal lesions other than minimal inflammatory cell infiltration - refer to Table 11 and Appendix E.

Phase III. Twenty-four animals comprising Phase III of the study were treated with "neutralized" CAIS to ascertain the vesicating potential of chemically degraded CAIS. Incidence/response data related to microvesication are summarized in Tables 12, 13, and 14. A summary of histopathologic changes, including vesication, is presented in Tables 15 and 16. Individual histopathology data appear in Appendix E. Eight animals were dosed with "archived" wastestreams, agent/chloroform solutions, and neat HD. Guinea-pigs dosed with HD and agent/chloroform solutions demonstrated at least minimal microvesication along with consistent, marked epidermal and follicular necrosis. The "Blue" process wastestream ("archived"; 25μL application) resulted in intermediate to severe microblisters and severe epidermal necrosis at all sites dosed (refer to Tables 12 and 16 and Appendix E). The impression of the pathologist reading the slides was that lesions did not appear to be "basal cell specific", as chemical blistering agents appear to cause, nor did the lesions resulting from application of the "Blue" wastestream penetrate deeply enough to cause severe necrosis in the follicular epithelium. A photomicrograph

TABLE 10. PHASE II. VESICATION (MICROBLISTER FORMATION) IN HAIRLESS GUINEA PIGS FOLLOWING DERMAL EXPOSURE TO HD, AGENT/$CHCl_3$ SOLUTIONS, OR NEUTRALIZING SOLUTION (DCDMH/$CHCl_3$/t-BuOH)[a]

Treatment Group[b]	Animal No.	Microblister Severity (0-4)							Response	Mean Severity
		301	305	306	309					
Neat HD (1 μL)		2	2	1[c]	3				4/4	2.0
10% HD/$CHCl_3$										
	50 μL	2	2						2/2	2.0
	10 μL	2	3	3	3				4/4	2.8
	5 μL			1[c]	0[c]				1/2	0.5
10% HN/$CHCl_3$										
	50 μL	2	2						2/2	2.0
	10 μL	2	2	2	4				4/4	2.5
	5 μL			4	4				2/2	4.0
10% L/$CHCl_3$										
	50 μL	4	3						2/2	3.5
	10 μL	3	3	3	4				4/4	3.3
	5 μL			4	4				2/2	4.0
Treatment Group[d]	Animal No.	312	316	311	313	315	317	324	Response	Mean Severity
Neat HD (1 μL)		3	3	3	2	2	2	3	7/7	2.6
10% HD/$CHCl_3$										
	10 μL	3	3						2/2	3.0
	5 μL	3	3	2	3	3	2	4	7/7	2.9
10% HN/$CHCl_3$										
	10 μL	4	3						2/2	3.5
	5 μL	3	4	3	4	2	3	4	7/7	3.3
10% L/$CHCl_3$										
	10 μL	3	4						2/2	3.5
	5 μL	3	4	3	4	4	2	4	7/7	3.4
DCDMH/$CHCl_3$/ t-BuOH	(20 μL)			0	0	0	0	0	0/5	0

a At 24 hr after dosing, animals were evaluated for skin injury, sacrificed, and skin samples taken and prepared for histopathology.
b Exposure duration 2 hr.
c Presence of ulcer prevented formation of microblister.
d Exposure duration 1hr.

TABLE 11. PHASE II. SUMMARY OF HISTOPATHOLOGY RESULTS

Experiment Date/ Animal ID	Test Article	Dose Volume (μL)	Time to Decon. (hr)	No.of Animals	No. of Sites	Number of Animals with Sign						
						Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
02/19/96 (301, 305)	10% $L/CHCl_3$	10	2	2	2	2	2	2	0	0	2	0
	10% $L/CHCl_3$	50	2	2	2	2	2	2	0	0	1	0
	10% $HN/CHCl_3$	10	2	2	2	2	2	2	0	0	0	0
	10% $HN/CHCl_3$	50	2	2	2	2	2	2	0	0	0	0
	10% $HD/CHCl_3$	10	2	2	2	2	2	2	0	0	1	0
	10% $HD/CHCl_3$	50	2	2	2	2	2	2	0	0	1	0
	Neat HD	1	2	2	2	2	2	2	0	0	0	0
02/21/96 (306, 309)	10% $L/CHCl_3$	5	2	2	2	2	2	2	0	1	1	0
	10% $L/CHCl_3$	10	2	2	2	2	2	2	0	1	1	0
	10% $HN/CHCl_3$	5	2	2	2	2	2	2	1	0	0	0
	10% $HN/CHCl_3$	10	2	2	2	2	2	2	1	1	0	0
	10% $HD/CHCl_3$	5	2	2	2	1	2	2	0	2	0	0
	10% $HD/CHCl_3$	10	2	2	2	2	2	2	1	1	0	0
	Neat HD	1	2	2	2	2	2	2	0	1	0	0

TABLE 11. PHASE II. SUMMARY OF HISTOPATHOLOGY RESULTS (CONT'D)

Experiment Date/ Animal ID	Test Article	Dose Volume (µL)	Time to Decon. (Hr)	No. Of Animals	No. of Sites	Number of Animals with Sign						
						Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
02/27/96 (312, 316)	10% $L/CHCl_3$	5	1	2	2	2	2	2	0	0	2	0
	10% $L/CHCl_3$	10	1	2	2	2	2	2	0	0	2	0
	10% $HN/CHCl_3$	5	1	2	2	2	2	2	2	0	0	0
	10% $HN/CHCl_3$	10	1	2	2	2	2	2	2	0	0	0
	10% $HD/CHCl_3$	5	1	2	2	2	2	2	1	1	0	0
	10% $HD/CHCl_3$	10	1	2	2	2	2	2	1	0	1	0
	Neat HD	1	1	2	2	2	2	2	1	0	0	0
03/05/96 (311, 313, 315, 317, 324)	10% $L/CHCl_3$	5	1	5	5	5	5	5	0	1	5	0
	10% $HN/CHCl_3$	5	1	5	5	5	5	5	4	1	1	0
	10% $HD/CHCl_3$	5	1	5	5	5	5	5	1	3	2	0
	Neutralizing Solution	20	1	5	20	0	0	0	0	0	0	0
	Neat HD	1	1	5	5	5	5	5	0	0	1	0

TABLE 12. PHASE III. VESICATION (MICROBLISTER FORMATION) IN HAIRLESS GUINEA PIGS FOLLOWING EXPOSURE TO "ARCHIVED" RRS WASTESTREAMS, AGENT/$CHCl_3$ SOLUTIONS, OR NEAT SULFUR MUSTARD (HD) [a,b]

Treatment Group		Microblister Severity (0-4)									
	Animal No.	494	496	497	499	310	491	493	498	Response	Mean Severity Score
Neat HD (1 μL)		3	2	2	3	1	2	2	1	8/8	2.0
10% HD/$CHCl_3$	(5 μL)	1	0	2	4	2	1	1	3	7/8	1.8
10% HN/$CHCl_3$	(5 μL)	3	0	1	4	4	4	4	2	7/8	2.8
10% L/$CHCl_3$	(5 μL)	4	1	4	3	1	4	2	3	8/8	2.8
"Blue" wastestream [c]	(25 μL)	2	4	2	2	2	3	4	3	8/8	2.8
"Red" wastestream [c]	(25 μL)	0	0	0	0	0	0	0	0	0/8	0
"Charcoal" wastestream [c]	(25 μL)	0	0	0	0	0	0	0	0	0/8	0

a Each animal was dosed percutaneously (1 hr exposure) with neat HD, agent/ $CHCl_3$ solution, and "archived" wastestreams. Sites were evaluated visually at about 24 hr after dosing, and the animals then sacrificed and skin samples taken and prepared for histopathologic evaluation.

b Dosing volumes of HD and agent/$CHCl_3$ solutions, as well as the duration of exposure, were based on preliminary tests. Dosing volumes of wastestreams were based upon approximate ratio of neutralization solution volume to volume of agent treated.

c Wastestreams were generated from the reaction of DCDMH (oxidant) with neat HD ("Blue" process), with 10% HD, HN or L in $CHCl_3$ ("Red" process), or with HD, HN, or L on charcoal ("Charcoal" process).

TABLE 13. PHASE III. MICROBLISTER FORMATION IN HAIRLESS GUINEA PIGS FOLLOWING EXPOSURE TO EQUAL VOLUMES OF "ARCHIVED" RRS WASTESTREAMS OR AGENT/$CHCl_3$ SOLUTIONS[a]

Treatment Group		Animal No. 383	385	389	400	Response	Mean Severity Score
		Microblister Severity (0-4)					
10% HD/$CHCl_3$	(10 μL)	2	3	2	3	4/4	2.5
10% HN/$CHCl_3$	(10 μL)	3	4	3	4	4/4	3.5
10% L/$CHCl_3$	(10 μL)	3	4	2	3	4/4	3.0
"Blue" wastestream[b]	(10 μL)	0	1	2	3	3/4	1.5
"Red" wastestream[b]	(10 μL)	0	0	0	0	0/4	0
"Charcoal" wastestream[b]	(10 μL)	0	0	0	0	0/4	0

a Each animal was dosed dermally (1 hr exposure) with agent/$CHCl_3$ solutions and wastestreams. Sites were evaluated visually at about 24 hr after dosing, and the animals then sacrificed and skin samples taken and prepared for histolopathologic evaluation.

b Wastestreams (product solutions) generated from reaction of oxidant (DCDMH) with HD - "Blue"; 10% HD, HN or L in $CHCl_3$ - "Red" ; HD, HN or L on charcoal - "Charcoal".

TABLE 14. PHASE III. MICROBLISTER FORMATION IN HAIRLESS GUINEA PIGS FOLLOWING EXPOSURE TO "FRESH" RRS WASTESTREAMS, AGENT/$CHCl_3$ SOLUTIONS, OR NEAT HD [a,b]

Treatment Group		Animal No.	339	341	342	346	340	345	351	352	Response	Mean Severity Score
			Microblister Severity (0-4)									
Neat HD	(1 μL)		2	2	3	2	0	1	1	2	7/8	1.6
10% HD/$CHCl_3$	(5 μL)		3	2	3	2	2	2	1	1	8/8	2.0
10% HN/$CHCl_3$	(5 μL)		3	2	4	4	3	1	1	2	8/8	2.5
10% L/$CHCl_3$	(5 μL)		4	3	3	2	3	3	4	3	8/8	3.1
"Blue" wastestream [c]	(25 μL)		2.5	1	2	1	3	1.5	2	1.5	8/8	1.8
"Red" wastestream [c]	(25 μL)		0	0	0.5 [d]	0	0	0	0	0	1/8	0

Treatment Group		Animal No.	379	380	387	388	Response	Mean Severity Score
10% HD/$CHCl_3$	(5 μL)		2	3	3	4	4/4	3.0
10% HN/$CHCl_3$	(5 μL)		3	4	2	3	4/4	3.0
10% L/$CHCl_3$	(5 μL)		4	4	4	4	4/4	4.0
"Charcoal" wastestream [e]	(25 μL)		0	0	0	0	0/4	0

a Each animal was exposed dermally for 1 hr to "test article" (neat HD and/or agent/$CHCl_3$ solution, and wastestreams). At 24 hr after dosing, animals were evaluated for gross skin injury and then sacrificed and skin samples taken and prepared for histopathologic evaluation.

b Dosing volumes and duration of exposure were determined from preliminary testing. Dosing volume of wastestreams was selected on the basis of approximate neutralization solution volume to volume of agent. Wastestreams were generated via the reaction of DCDMH with neat HD - "Blue"; HD, HN, or L in $CHCl_3$ -"Red"; and HD, HN, or L on charcoal - "Charcoal".

c Mean value for the two sites dosed with each wastestream on each animal.

d Could be due to adjacent HD-treated site.

e Three sites on each animal were dosed with "Charcoal" wastestream and no sites exhibited microblisters.

TABLE 15. PHASE III. SUMMARY OF HISTOPATHOLOGY RESULTS

Date, Source of Wastestream	Agent / Compound[e]	Dose Volume (µL)	No. Of Animals	No.of Sites	Number of Animals with Sign: Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
03/13/96, 03/21/96 "Archived" Wastestreams	L	5	8	8	8	8	8	0	6	5	0
	HN	5	8	8	7[a]	8	8	3	4	0	0
	HD	5	8	8	7[a]	8	8	3	7	0	0
	"Red" Wastestream	25	8	8	0[b,c,d]	1[b,c,d]	0[b,c,d]	2	0[b,d]	0	0
	"Blue" Wastestream	25	8	8	8	8	1[b,c,d]	1	1[d]	0	0
	"Charcoal" Wastestream	25	8	8	0[b,c,d]	6	0[b,c,d]	2	0[b,d]	0	0
	Neat HD	1	8	8	8	8	8	0	7	1	0
06/20/96, 06/26/96 "Fresh" Wastestreams	L	5	8	8	8	8	8	1	3	5	0
	HN	5	8	8	8	8	8	2	5	2	0
	HD	5	8	8	8	8	8	2	5	5	0
	"Red" Wastestream	25	8	16	1[b,c,d]	2[b,c,d]	1[b,c,d]	1	0	0	0
	"Blue" Wastestream	25	8	16	8	8	7	2	1	0	0
	Neat HD	1	8	8	7	8	8	0	5	5	0
08/13/96 "Archived" Wastestreams	L	10	4	4	4	4	4	0	0	4	1
	HN	10	4	4	4	4	4	4	1	2	0
	HD	10	4	4	4	4	4	1	0	2	0
	"Red" Wastestream	10	4	4	0	0	0	0	0	0	0
	"Blue" Wastestream	10	4	4	3	3	2	1	0	1	0
	Charcoal Wastestream	10	4	4	0	1	0	1	0	0	0
08/29/96 "Fresh" Wastestreams	L	5	4	4	4	4	4	0	1	4	0
	HN	5	4	4	4	4	4	1	1	1	0
	HD	5	4	4	4	4	4	0	1	2	0
	"Charcoal" Wastestream	25	4	12	0	4	4	1	0	0	0

Note: All times to decontamination were 1 hr.
a Marked ulceration at the dosing site on animal number 496 obscured any evidence of microvesication.
b Incidence of sign was significantly less than that for sites dosed with L using McNemar's Test and a significance level of p=0.05.
c Incidence of sign was significantly less than that for sites dosed with HN using McNemar's Test and a significance level of p=0.05.
d Incidence of sign was significantly less than that for sites dosed with HD using McNemar's Test and a significance level of p=0.05.
e Agent (L, HN, HD) at a concentration of 10% in chloroform.

TABLE 16. PHASE III. SUMMARY OF INTERMEDIATE TO SEVERE HISTOPATHOLOGY RESULTS

Date, Source of Wastestream	Agent / Compound[e]	Dose Volume (µL)	No. Of Animals	No.of Sites	Number of Animals with Sign Rated Intermediate to Severe						
					Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
03/13/96, 03/21/96 "Archived" Wastestreams	L	5	8	8	6[a]	8	8	0	6	2	0
	HN	5	8	8	6[a]	8	8	0	3	0	0
	HD	5	8	8	4[a]	8	8	0	7	0	0
	"Red" Wastestream	25	8	8	0[b,c]	0[b,c,d]	0[b,c,d]	0	0[b,d]	0	0
	"Blue" Wastestream	25	8	8	8	8	0[b,c,d]	0	0[b,d]	0	0
	"Charcoal" Wastestream	25	8	8	0[b,c]	2[b,c,d]	0[b,c,d]	0	0[b,d]	0	0
	Neat HD	1	8	8	6[a]	8	8	0	6	0	0
06/20/96, 06/26/96 "Fresh" Wastestreams	L	5	8	8	8	8	8	0	3	2	0
	HN	5	8	8	6[a]	8	8	0	3	1	0
	HD	5	8	8	6[a]	8	8	0	5	2	0
	"Red" Wastestream	25	8	16	0[b,c,d]	1[b,c,d]	1[b,c,d]	0	0	0	0
	"Blue" Wastestream	25	8	16	7	8	2[b,c,d]	0	1	0	0
	Neat HD	1	8	8	5	8	8	0	5	1	0
08/13/96 "Archived" Wastestreams	L	10	4	4	4	4	4	0	0	4	0
	HN	10	4	4	4	4	4	0	0	0	0
	HD	10	4	4	4	4	4	0	0	1	0
	"Red" Wastestream	10	4	4	0	0	0	0	0	0	0
	"Blue" Wastestream	10	4	4	2	2	0	0	0	0	0
	"Charcoal" Wastestream	10	4	4	0	0	0	0	0	0	0
08/29/96 "Fresh" Wastestream	L	5	4	4	4	4	4	0	1	3	0
	HN	5	4	4	4	4	4	0	0	0	0
	HD	5	4	4	4	4	4	0	1	1	0
	"Charcoal" Wastestream	25	4	12	0	0	0	0	0	0	0

Note: All times to decontamination were 1 hr.
a Ulceration at some dosing sites may have obscured evidence of microvesication.
b Incidence of sign was significantly less than that for sites dosed with L using McNemar's Test and a significance level of $p=0.05$.
c Incidence of sign was significantly less than that for sites dosed with HN using McNemar's Test and a significance level of $p=0.05$.
d Incidence of sign was significantly less than that for sites dosed with HD using McNemar's Test and a significance level of $p=0.05$.
e Agent (L, HN, HD) at a concentration of 10% in chloroform.

representative of the morphologic changes observed following treatment with a vesicant is shown in Figure 2a, and one demonstrating the appearance of normal hairless guinea pig epidermis is shown in Figure 2b. The morphologic changes seen consist of ballooning degeneration and loss of epidermal basal cell attachment to the underlying basement membrane. Neither "Red" nor "Charcoal" process wastestreams ("archived"; 25μL application) produced microblisters (Tables 12 and 15). The "Red" process wastestream produced only minimal pustular epidermitis or minimal epidermal necrosis (refer to Table 15 and Appendix E). The "Charcoal" process wastestream ("archived"; 25μL application) killed some surface epithelial cells (minimal to intermediate epidermal necrosis) but did not penetrate to basal cells - refer to Table 15 and Appendix E. Four guinea pigs were dosed with 10 μL of "Blue", "Red", and "Charcoal" process wastestreams ("archived") and evaluated for dermal effect. The "Blue" process wastestream induced microblisters whereas the "Red" and "Charcoal" process wastestreams did not elicit microblister formation. The findings are highlighted in Table 13. Histopathology findings are summarized in Tables 15 and 16, and individual histopathology data are presented in Appendix E.

"Fresh" wastestream-induced skin effects were also evaluated. Data on microvesication are presented in Tables 14, 15, and 16, and other histopathologic skin effects data are given in Tables 15 and 16. Individual animal histopathology results are presented in Appendix E. All agent-dosed sites (neat HD and agent/chloroform solutions) and all "Blue" process wastestream sites demonstrated histopathologic lesions including microvesication. In "fresh" "Red" process wastestream-dosed animals, minimal to no lesions were seen on histopathologic examination. One "Red" process wastestream site in one animal demonstrated histopathology, including minimal microvesication; however, this lesion was incompatible with what had been noted previously. The "Charcoal" process wastestream did not produce microblisters and none of the sites demonstrated histopathology graded more than minimal.

3.3 Data Analysis Results

3.3.1 Gross Pathology (Erythema and Edema)

Means and standard deviations were calculated for erythema and edema scores (Phase II and III Studies) and for lesion areas (Phase III Studies). Analysis of variance was performed for

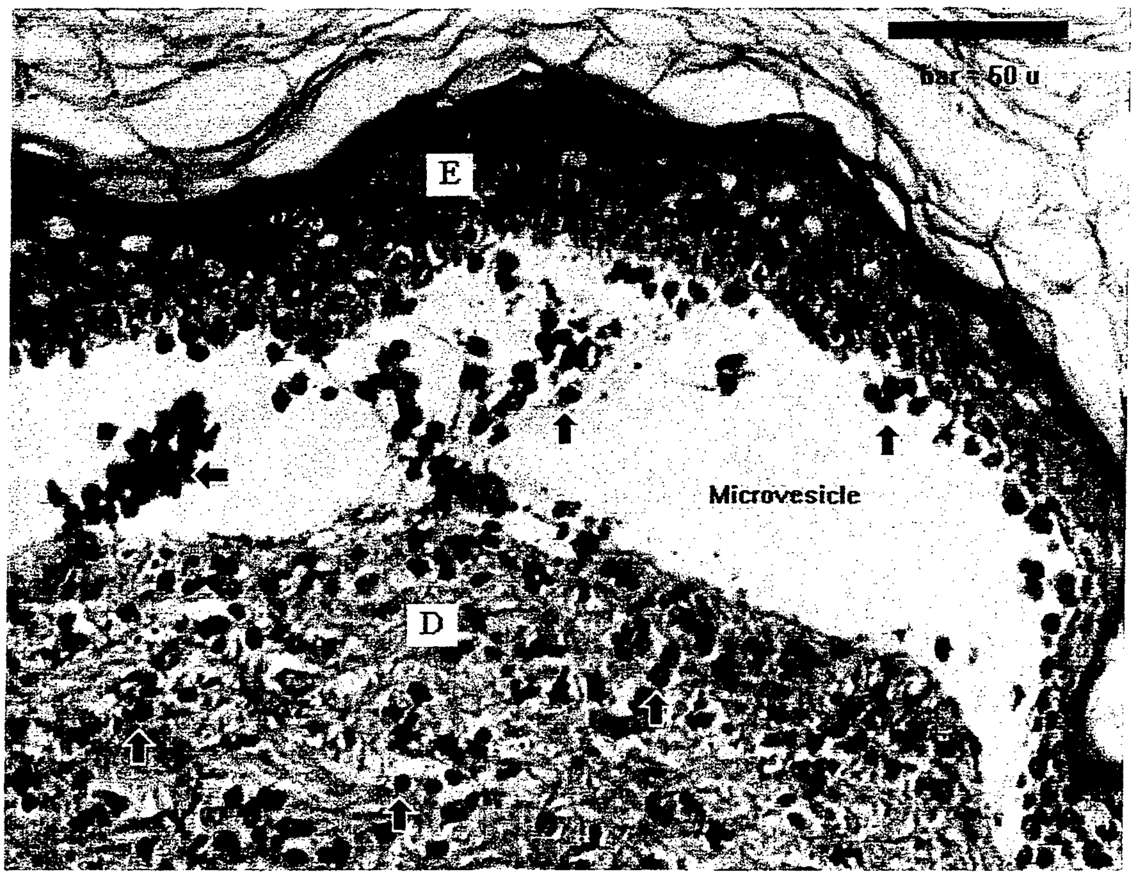

Fig. 2a. Typical microblister in a hairless guinea pig 24 hours after exposure to vesicant. Epidermis (E) is eosinophilic and shrunken due to necrotic epithelium; dermis (D) is also necrotic and contains an infiltrate of polymorphonuclear cells (arrows), as does the microblister cavity (microvesicle).

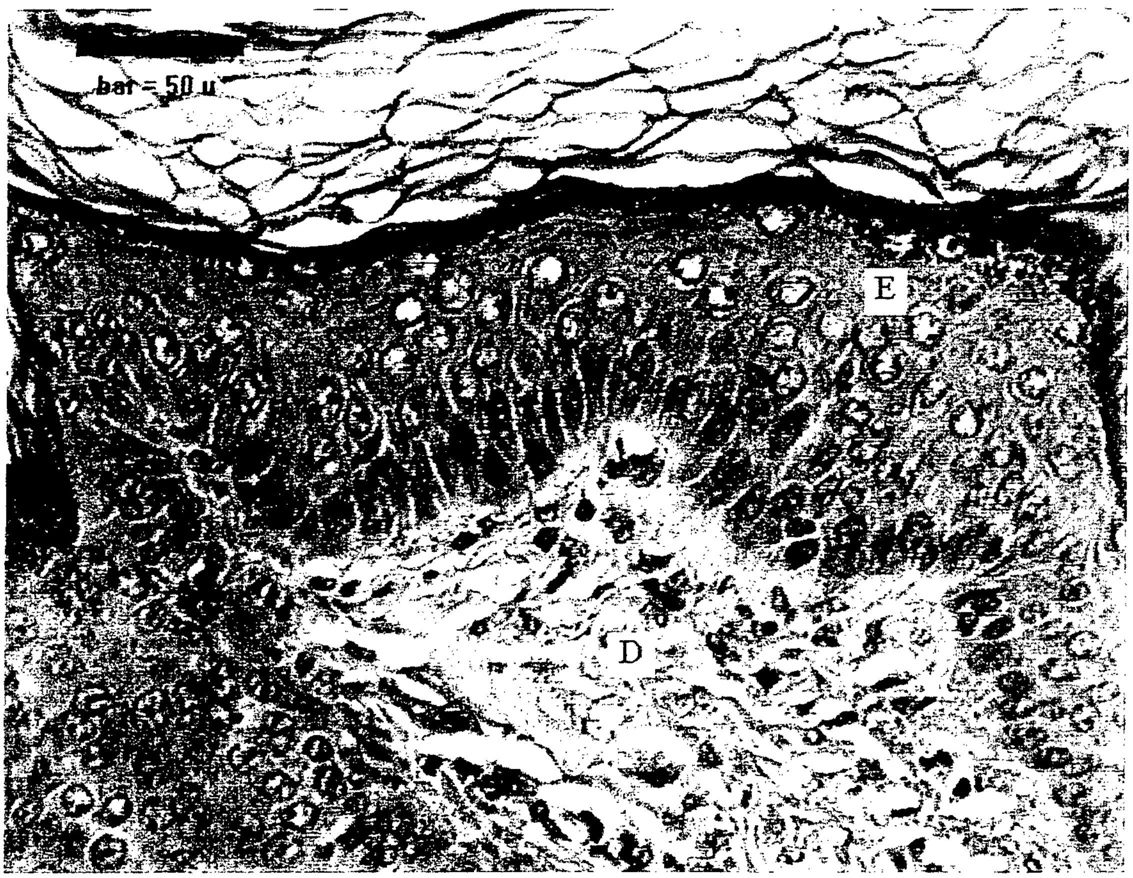

Fig. 2b. Normal skin from a hairless guinea pig. Epidermis (E) and dermis (D) are visible. Note differences in appearance from the necrotic tissue depicted in Fig. 2a. Magnification of both skin photomicrographs is the same.

inflammation scores and lesion areas. Tables 8 and 9 present means and standard deviations for erythema and edema scores. Significant decreases in average inflammation scores resulted when comparing wastestream-dosed ("archived" or "fresh" -25 μL volume application) to agent-dosed sites (HD or agent/chloroform) - refer to Table 9. Some significant increases in lesion areas were noted with wastestreams, presumably due to the larger volume dosed. For the August 13, 1996 experiment (vesicancy assay of "archived" wastestreams), significant decreases in average inflammation scores as well as average lesion areas resulted when comparing wastestream-dosed ("archived" "Red" and "Blue" process wastestreams - 10 μL volume applications of wastestreams and agent/chloroform solutions) to agent-dosed sites. All observed inflammation scores and lesion areas from the "fresh" "Charcoal" wastestream-dosed sites were zero.

3.3.2 Histopathology

Statistical analysis (McNemar's test) of the histopathology data was performed to ascertain the significance between treatment groups (neat HD, agent/chloroform solutions, and wastestreams) at the 0.05 significance level. Sites dosed with "Red" or "Charcoal" wastestream ("archived", 25 μL volume application) exhibited a <u>significant decrease</u> in incidence of <u>microblisters</u> compared to those sites dosed with HD or agent/chloroform solutions. Sites dosed with the wastestreams also showed a significant decrease in the incidence of follicular necrosis compared to sites dosed with any of the three agents (HD, HN, or L in chloroform; neat HD). Some significant neutralized wastestream versus agent differences also resulted with respect to incidence of epidermal and dermal necrosis.

Sites dosed with "Red" wastestream ("fresh", 25 μL volume application) showed a <u>significant decrease</u> in incidence of <u>microblisters</u>, epidermal necrosis, and follicular necrosis compared to that on sites dosed with any of the three agents. Numerical reductions in some pathology from wastestream-dosed sites ("archived", 10 μL volume application) were observed, although they were not statistically significant due to the smaller number of animals tested.

Statistical analysis of incidence of intermediate to severe histopathologic signs was also performed. Sites dosed with "Red" or "Charcoal" wastestream ("archived", 25 μL volume application) demonstrated a <u>significant decrease</u> in incidence of microblisters compared to that on sites dosed with L/chloroform and HN/chloroform. A decrease in incidence was also observed for the “Red” or “Charcoal” wastestream compared to that on sites dosed with HD/chloroform, but were not statistically significant because only four of the eight animals exposed to HD/chloroform had intermediate to severe microblisters. Sites dosed with “Red” or “Charcoal” wastestream (“archived”, 25 μL volume application) demonstrated a significant decrease in incidence of epidermal necrosis and follicular necrosis compared to that on sites dosed with any of the three agents. Sites dosed with “Red” wastestream (“fresh”, 25 μL volume application) showed a significant decrease in incidence of microblisters, epidermal necrosis, and follicular necrosis compared to that on sites dosed with any of the three agents. Sites dosed with “Blue” wastestream showed a significant decrease in incidence of follicular necrosis compared to that observed on sites dosed with any of the three agents.

Sites dosed with "fresh" "Charcoal" wastestream (25 μL volume application) exhibited a numerical reduction in incidence of microblisters, although this was not statistically significant due to the smaller number of animals tested, compared to that observed on sites dosed with any of the three agents. Statistical analyses also were conducted on the pooled "Charcoal" wastestream data ("fresh" and "archived", 25 μL volume applications- see Table 17). These analyses assumed that the probability of a microblister and other histopathologic endpoints is similar for sites dosed with "archived" and "fresh" "Charcoal" wastestreams. Pooled data for sites dosed with "Charcoal" wastestream showed a significant decrease in incidence of microblisters and follicular necrosis compared to that on sites dosed with any of the three agents. Statistical analyses of incidence of intermediate to severe histopathologic signs (Table 18) were also performed on the pooled "Charcoal" wastestream data ("fresh" or "archived", 25 μL volume application). Sites dosed with "Charcoal" wastestream showed a significant decrease in incidence of intermediate to severe microblisters, epidermal necrosis, and follicular necrosis compared to that observed on sites dosed with any of the three agents.

TABLE 17. PHASE III. SUMMARY OF HISTOPATHOLOGY FOLLOWING DOSING OF "CHARCOAL" WASTESTREAMS[a]

Agent/ Compound[f]	Dose Volume (µL)	No. Of Animals	No. of Sites	Number of Animals with Histopathology						
				Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
L	5	12	12	12	12	12	0	7	9	0
HN	5	12	12	11[b]	12	12	4	5	1	0
HD	5	12	12	11[b]	12	12	3	8	2	0
"Charcoal" Wastestream	25	12	20	0[c,d,e]	10	4[c,d,e]	3	0[c,e]	0[c]	0

Note: All times to decontamination were 1 hr.
a Pooled data from the "Charcoal" wastestream received 1/25/96 and dosed on 3/13 and 3/21/96 and wastestream received 8/29/96 and dosed the same day. Volume of "Charcoal" wastestream dosed was 25 µL at each site.
b Marked ulceration at the dosing site on animal #496 may have obscured microvesication.
c Incidence of pathology was significantly less than that for sites dosed with L based on McNemar's Test at the 0.05 significance level.
d Incidence of pathology was significantly less than that for sites dosed with HN based on McNemar's Test at the 0.05 significance level.
e Incidence of pathology was significantly less than that for sites dosed with HD based on McNemar's Test at the 0.05 significance level.
f Agent (L, HN, HD) at a concentration of 10% in chloroform.

TABLE 18. PHASE III. SUMMARY OF INTERMEDIATE TO SEVERE HISTOPATHOLOGY FOLLOWING DOSING OF "CHARCOAL" WASTESTREAM[a]

Agent/ Compound[e]	Dose Volume (µL)	No. of Animals	No. of Sites	Number of Animals with Histopathology Rated Intermediate to Severe						
				Micro-blister	Epidermal Necrosis	Follicular Necrosis	Pustular Epidermitis	Dermal Necrosis	Hemorrhage	Vascular Necrosis
L	5	12	12	10	12	12	0	7	5	0
HN	5	12	12	10	12	12	0	3	0	0
HD	5	12	12	8	12	12	0	8	1	0
"Charcoal" Wastestream	25	12	20	0[b,c,d]	2[b,c,d]	0[b,c,d]	0	0[b,d]	0	0

Note: All times to decontamination were 1 hr.
a Pooled data from the "Charcoal" wastestream received 1/25/96 and dosed 3/13 and 3/21/96 and wastestream received 8/29/96 and dosed the same day.
b Incidence of pathology was significantly less than that for sites dosed with L based on McNemar's Test at the 0.05 significance level.
c Incidence of pathology was significantly less than that for sites dosed with HN based on McNemar's Test at the 0.05 significance level.
d Incidence of pathology was significantly less than that for sites dosed with HD based on McNemar's Test at the 0.05 significance level.
e Agent (L, HN, HD) at a concentration of 10% in chloroform.

4. Discussion

The intent of the process chemistries was to develop neutralization reactions that achieved destruction of CAIS agents, forming wastestreams with minimal toxic hazards. Achieving the desired objectives represented a formidable challenge since chemical reactions with the agents can result in the formation of reaction products/by-products having vesicant action and/or a high degree of systemic toxicity. Destruction of agents involves complex chemical reactions, which is certainly the case for sulfur mustard. HD destruction is complicated by the presence of sulfur and chlorine in the HD molecule, which in some cases facilitates and in others impedes the chemical degradation of HD. Possible methods suggested for agent destruction have included oxidation and chlorination for HD and oxidation for nitrogen mustard and lewisite. The toxicity of the degradation products resulting from the chemical neutralization of HD, HN, or L is of concern to the toxicology, health, and regulatory communities. The current studies were undertaken to assess the vesicant properties of neutralized CAIS.

Current methods for demilitarizing CAIS are still based largely on chemical neutralization via oxidizing materials although the use of DCDMH as oxidant does provide an alternative degradation pathway via the chlorination of HD. The oxidation of sulfur mustard, as pointed out by Franke (1967), represents one of the most important decontamination reactions for HD. The oxidation of sulfur mustard via various oxidizers (e.g., hydrogen peroxide, hypochloric acid and its salts, potassium permanganate, nitric acid, DCDMH, etc.) yields various compounds whose composition depends on the nature of the oxidant used and the specific reaction conditions. Most easily formed is HD sulfoxide which on oxidation yields HD sulfone - both represent major oxidation products of sulfur mustard.

The oxidation of HD not only alters the skin-damaging properties of HD but the systemic toxicity of sulfur mustard as well. The oxidation of HD is of great interest since sulfoxide formation, on chemical neutralization of HD, can be considered a "detoxification". The "detoxification" of HD via oxidation to the sulfoxide was demonstrated in the 1940's. In contrast, the formation of mustard sulfone, a product of further oxidation, can contribute to an enhanced systemic toxicity and vesicant potential of the product solution/mixture. HD sulfone, having the

$S(O)_2$ functional group, is highly poisonous and comparable in toxicity to HD.[6] Research conducted since Philips' review (Philips, 1950) on sulfur mustard pharmacology/toxicology demonstrated that HD sulfone is a highly toxic vesicant.

Certainly, based on the known toxicity characteristics of mustard sulfone, mustard sulfoxide, and their vinyl derivatives, it is crucial that the process chemistries developed for the destruction of CAIS employ oxidants that minimize the formation of HD sulfone and HD analogs having comparable biological activity (systemic toxicity and vesicancy) to that of HD.

A concern regarding the vesication potential of HD degradation products/by-products prompted a review of the toxicology literature pertaining to sulfur mustard products/by-products information which is summarized in Table 19. The reader is referred to a review on the subject matter (Olajos *et al.* 1996).

For purposes of this report, discussion on the relationship between chemical structure and vesication is limited to the thioether molecule. Degradation product(s) of nitrogen mustards have not been implicated as having vesicant potential although this area of research needs to be explored. The principal degradation product of lewisite, namely L oxide, is a potent vesicant. The reader is referred to several papers/reviews on the subject of mustard vesication and toxicology (Bouder, 1940; Anslow and Houck, 1946; Philips, 1950; Aleksandrov, 1969; Franke, 1967; and Henry, 1991) as well as reviews covering the systemic toxicity and pathology of nitrogen mustards (Anslow and Houck, 1946; Renshaw, 1946; Cope *et al.*, 1946; and Graef *et al.*, 1948). The subject of lewisite toxicology and pathology has also been amply covered (Wardell, 1941; Gates *et al.*, 1946; and Goldman and Dacre, 1989).

The vesicant potential of sulfur mustard derivatives (oxidation and chlorination products) has been investigated since the 1920's to modern times. Research has indicated that the strongest vesicant action is exerted by β-halogenated sulfides. The position and degree of chlorination influences the vesicant potential of the thioether molecule. With respect to the site of chlorination, Kirner (1928) and Dawson and Wardell (1930) concluded that compounds having

[6] HD is easily destroyed by all chlorinating agents (aqueous or anhydrous medium). Under appropriate conditions, the chlorination of HD can proceed to form various polychlorides. In the presence of water, chlorination of HD is altered resulting in the formation as sulfoxides (Aleksandrov, 1969).

the chlorine atom in the beta position were considerably more vesicant that those having chlorine in the alpha or gamma position. The degree of chlorination also influences the vesicant activity of the sulfide molecule and hence the early use of chlorination to degrade HD. Monosubstitution analogs of HD, regardless of position, are less effective vesicants than HD. As previously stated, the introduction of halogen atoms results in decreased toxicity and markedly diminished vesicant action. Research in the 1920s (Mann and Pope, 1922; Peters and Walker, 1923; and Lawson and Dawson, 1927) - summarized by Bouder (1940) - indicated that the higher chlorinated derivatives (e.g., tri-, tetra-, and hexachloro derivatives) of HD (saturated or unsaturated) were non-vesicant. A summary of the vesicant potential of various chlorinated analogs of sulfur mustard are given in Table 20. The demilitarization of CAIS as stated is based on chemical neutralization via oxidizing materials which not only alters the systemic toxicity of HD (as discussed) but the skin damaging properties (irritation, vesication). Fuson *et al.* (1943) on review of the vesicant activity of sulfur compounds concluded that compounds containing the S(0) group were non-vesicant. Mustard sulfone, containing the S $(0)_2$, functional group is a known vesicant (vesicancy potential 1/7 to 1/5 of HD; Bergmann *et al.*, 1945). The formation of HD sulfone can contribute to an enhanced vesicant potential of the product solution/mixture (wastestream).

TABLE 19. SYNOPSIS OF DERMAL TOXICITY DATA FOR CAIS AGENTS, AGENT DEGRADATION PRODUCTS, RRS OXIDANTS AND SOLVENTS[a]

Compound	Dermal Toxicity[b] (LD_{50}/LDLo/TDLo)	References	Skin Effects (Irritation, Vesication)[b]	References
AGENTS				
HD [bis(2-chloroethyl)sulfide]	LD_{50} (40-100 mg/kg)	Anslow & Houck (1946)	Severe irritant/escharotic, severe vesicant	Marshall & Williams (1921); Gates & Moore (1946); Renshaw (1946)
L [dichloro(2-chlorovinyl)arsine]	LD_{50} (5-6 mg/kg)	Cameron et al. (1946); Gates et al. (1946)	Severe irritant/escharotic, severe vesicant	Gates et al. (1946)
HN-1 [bis(2-chloroethyl)ethylamine]	LD_{50} (15-20 mg/kg)	Smith (1943a) ; Anslow & Houck (1946)	Severe irritant/escharotic, severe vesicant	Cope et al. (1946); Renshaw (1946)
HN-3 [tris(2-chloroethyl)amine]	LD_{50} (5-20 mg/kg)	Smith (1943d) ; Anslow & Houck (1946)	Severe irritant/escharotic, severe vesicant	Cope et al. (1946); Renshaw (1946);
OXIDIZED DERIVATIVES				
HD sulfoxide	(-)[c]	(-)[c]	Irritant, non-vesicant	Marshall & Williams (1921); Lawson & Dawson (1927); Young et al. (1944)
Sulfoxide, 2-chloroethyl vinyl	(-)[d]	(-)[d]	Irritant, non-vesicant	Thomson et al. (1945)
Divinyl sulfoxide	(-)[e]	(-)[e]	Irritant, non-vesicant	Fuson et al. (1943); Young et al. (1944) Thomson et al. (1945)
HD sulfone	(-)[f]	(-)[f]	Irritant/escharotic, vesicant	Marshall & Williams 1921); Young et al. (1944)

TABLE 19. (Continued)

Compound	Dermal Toxicity[a] (LD_{50}/LDLo/TDLo)	References	Skin Effects (Irritation, Vesication)[b]	References
OXIDIZED DERIVATIVES (Cont.)				
Sulfone, 2-chloroethyl vinyl	(-)[g]	(-)[g]	Irritant/escharotic, vesicant	Young et al. (1944) Thomson et al. (1945)
Divinyl sulfone	LD_{50} (≈ 20 mg/kg)	Smyth et al. (1962)	Irritant/escharotic, vesicant	Young et al. (1944); Thomson et al. (1945)
HN-1 oxide	(-)[h]	(-)[h]	(-)[n]	(-)[n]
HN-3 oxide	(-)[i]	(-)[i]	(-)[n]	(-)[n]
Lewisite oxide	(-)[j]	(-)[j]	Irritant/escharotic, vesicant	Young et al. (1944); Thomson et al. (1945)
2-chlorovinylarsonic acid	(-)[k]	(-)[k]	Irritant, non-vesicant	Young et al. (1944); Thomson et al (1945)
2-chlorovinylarsonous acid	(-)[l]	(-)[l]	Irritant, non-vesicant	Cameron et al. (1946)
OXIDIZERS				
DCDMH	LD_{50} (>20 g/kg)	EPA 8EHQ0281-0382; EPA 88-8100-228	Severe irritant	EPA 8EHQ0281-0382; EPA #88-8100-173 (cited in RTECS)

TABLE 19. (Continued)

Compound	Dermal Toxicity[a] (LD50/LDLo/TDLo)	References	Skin Effects (Irritation, Vesication)[b]	References
SOLVENTS				
Chloroform	LD_{50}(>20 g/kg)	NTIS AD-A062-138 (cited in RTECS)	Mild irritant	Guido and Martins (1988)
t-butyl alcohol	(-)[m]	(-)[m]	Mild irritant	Oettel (1936)

[a] Table modified from that originally compiled by Olajos et al., 1996.
[b] Rabbit as animal model unless otherwise indicated. Tests for irritancy based on animal and/or human studies. Test for vesicant action of agents conducted on human subjects.
[c] Mouse s.c. LD_{50} (>25 mg/kg) [Anslow and Houck (1946)].
[d] Rat oral (100 mg/kg, mortality 1/1) [Young et al., 1944]
[e] Mouse s.c. LD_{50} (>25 mg/kg) [Anslow and Houck (1946)].
[f] Mouse s.c. LD_{50} (>25 mg/kg) [Anslow and Houck (1946)].
[g] Acute toxicity undetermined.
[h] Mouse i.p. LD_{50} (50-100 mg/kg) [Bergmann and Fruton (1943); Stahmann and Bergmann (1946a)].
[i] Mouse i.p. LD_{50} (2-5 mg/kg) [Bergmann and Fruton (1943); Stahmann and Bergmann (1946a)].
[j] Mouse s.c. (mortalities: 2 mg/kg (0/5); 5 mg/kg (5/5); 10 mg/kg (5/5) [Young et al. (1944)].
[k] Mouse i.p. [mortalities: (1000 mg/kg 10/10; 500 mg/kg 0/10] (Young et al., 1944)
[l] Reported as hightly toxic, details not given (Cameron et al., 1946)
[m] Rabbit oral LDLo (4.5 g/kg) [RTECS].
[n] Young et al., (1944) reported HN2 oxide as non-vesicant; no data for HN1, HN3.

TABLE 20. VESICATION POTENTIAL OF VARIOUS ANALOGS/DERIVATIVES OF SULFUR MUSTARD[a]

Analogs/Derivatives (Saturated and Unsaturated)	Vesicant Activity	References[b]
OXIDIZED DERIVATIVES		
Mustard Sulfone (sulfone, bis(2-chloroethyl)	(POS)	Marshall & Williams (1921), Young *et al.* (1944)
Sulfone, 2-chloroethyl vinyl	(POS)	Young *et al.* (1944)
Divinyl Sulfone	(POS)	Young *et al.* (1944), Thomson *et al.* (1945)
Mustard Sulfoxide (sulfoxide, bis(2-chloroethyl)	(NEG)	Marshall & Williams (1921) Lawson & Dawson (1927) Fuson *et al.* (1943) Bergmann *et al.* (1945)
Divinyl Sulfoxide	(NEG)	Young *et al.* (1944) Thompson *et al.* (1945) Bergmann *et al.* (1945)
β-chloroethyl vinyl sulfoxide	(NEG)	Young *et al.* (1944)
α, β', -trichlorodiethyl sulfoxide	(NEG)	Young *et al.* (1944)
CHLORINATED DERIVATIVES		
bis(α-chloroethyl) sulfide	(NEG)	Peters and Walker 1923) Baldwin *et al.* (1924) Kirner (1928) Dawson & Wardell (1930)
α, β, β' -trichlorodiethyl sulfide	(NEG)	Mann & Pope (1922) Lawson & Dawson (1927)
α, β, β, β' tetrachlorodiethyl sulfide	(NEG)	Mann & Pope (1922) Lawson & Dawson (1927)
α, α^1, β, β' tetrachlorodiethyl sulfide	(NEG)	Lawson & Dawson (1927)
α, α β, β, β, β' hexachlorodiethyl sulfide	(NEG)	Mann & Pope (1922) Lawson & Dawson (1926) Dawson & Wardell (1930)
β-chloroethyl α, β dichlorovinyl sulfide	(NEG)	Lawson & Dawson (1926) Kirner (1928) Dawson & Wardell (1930)
β-chloroethyl α, β, β' trichlorovinyl sulfide	(NEG)	Lawson & Dawson (1926) Kirner (1928) Dawson & Wardell (1930)
β-chloroethyl chlorovinyl sulfide (α and β isomers)	(POS)	Lawson & Dawson (1926) Dawson & Wardell (1930) Fuson *et al.* (1943)

(a) Table from Olajos *et al.*, 1996
(b) citations are primary and/or secondary

The lack of vesicancy following treatment with "Red" and "Charcoal" process wastestreams is indicative of the effectiveness of the neutralization chemistries in destruction of chemical agent concomitant with the minimization of potentially vesicant-inducing products/by-products. Product analyses corroborated the results of the bioassay. The composite agent (HD, HN and L) levels in "archived" and "fresh" "Red" wastestreams and in "archived" and "fresh" "Charcoal" wastestreams did not elicit vesication in the volumes dosed.

Treatment with "Blue" process wastestreams ("archived" and "fresh") resulted in a vesicant response. The bioassay results were unexpected since the agent residual level was 15 ppm or less, a level below that expected to elicit a vesicant response. The most plausible explanation for vesication is that degradation product(s)/by-product(s) were present in the wastestreams and elicited the vesicant response. The association of vesicancy with the "Blue" process wastestreams ("archived" and "fresh") presents concerns regarding (1) unattained reduction in agent characteristics of the "Blue" wastestreams and (2) needed refinement of the analytical techniques for product identification in the wastestreams.

5. Conclusions

Based on the findings of these studies the following conclusions can be made.

- The vesicating properties of the "Blue" wastestream were not significantly reduced from that of the untreated CAIS (neat HD) prior to treatment with neutralization solution.
- The vesicating properties of both "Red" and "Charcoal" wastestreams, in the volumes dosed, were significantly lower than the untreated CAIS agent solutions.

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APPENDIX A

Study Protocol
Battelle SOP MREF II-009
Deviation Reports/Memos to File

Study Performed by Battelle Memorial Institute
Medical Research and Evaluation Facility
505 King Avenue, Building JM-3, Columbus, OH 43201-2693

STUDY TITLE:

Evaluation of the Vesicating Properties of Neutralized Chemical Agent Identification Set (CAIS) Components

PRINCIPAL INVESTIGATOR: Carl T. Olson 11/29/95

Carl T. Olson, DVM, ~~PhD~~, Study Director

SCIENTIFIC REVIEW: John B. Johnson

John B. Johnson, DVM, Manager, Medical Research and Evaluation Facility (MREF)

ATTENDING/CONSULTING VETERINARIAN: T. Peace

Tracy A. Peace, DVM, Study Veterinarian

STATISTICAL REVIEW: Ronald G. Menton

Ronald G. Menton, PhD, Study Statistician

CONTRACTING OFFICER'S REPRESENTATIVE:

Richard R. Stotts 29 Nov 95

Richard R. Stotts, LTC, USA, VC

Appendix A

PROTOCOL TITLE: Evaluation of the Vesicating Properties of Neutralized Chemical Agent Identification Set (CAIS) Components

PRINCIPAL INVESTIGATOR: Carl T. Olson, DVM, PhD

CO-INVESTIGATOR(S):

<u>Study Supervisor</u>: Robyn C. Kiser, B.S.

<u>Statistician</u>: Ronald G. Menton, Ph.D.

<u>Study Veterinarian</u>: Tracy A. Peace, D.V.M.

<u>Study Pathologist</u>: Allen W. Singer, D.V.M.

<u>Study Chemist</u>: Timothy L. Hayes, B.A.

<u>Sponsor</u>: Program Manager for Non-Stockpile Chemical Materiel (PMNSCM), USACMDA

<u>Sponsor Monitor</u>: LTC Richard R. Stotts, D.V.M., U.S. Army Medical Research Institute of Chemical Defense (USAMRICD)

I. NON-TECHNICAL SYNOPSIS:

The objective of this study is to assess the vesicating potential of wastestreams following neutralization of chemical agents HD, HN, and L contained in Chemical Agent Identification Sets (CAIS). Experiments are conducted with the hairless guinea pig to evaluate the vesicating properties of wastestreams and to develop a data base to address regulatory concerns of worker safety, industrial hygiene, and transportation and disposal of hazardous waste. This study will be conducted following the guidelines of the Environmental Protection Agency (EPA) Good Laboratory Practice (GLP) Standards.

II. BACKGROUND:

A. Background:

The Program Manager for Non-Stockpile Chemical Materiel has a requirement to develop and field a transportable system to neutralize vesicating (blister) agents contained in CAIS. Within these sets are glass ampules containing either 1) neat sulfur mustard (HD), 2) 5-10 percent HD, nitrogen mustard (HN), or Lewisite (L) in chloroform, or 3) 43 percent by weight HD, HN or L in a charcoal matrix. The proposed operation for neutralization of these vesicating agents consists of removing ampules from containers and crushing them in a neutralization solution under engineering controls. The neutralization solution is 0.555 M 1,3-dichloro-5,5-dimethylhydantoin in approximately 50:50 chloroform/t-butanol with about 3 percent water. Volume of neutralization solution to volume of ampules is approximately 20:1 for neat HD, 4:1 for agents in chloroform, and 70:1 for agents on the charcoal matrix. After chemical neutralization of the agents, the wastestreams will be turned over to a hazardous waste disposal contractor for ultimate disposal by incineration. The intent is to have wastestreams handled in a manner similar to industrial wastes that are readily transported and destroyed in accordance with regulatory guidelines. In order to be able to handle the wastestreams as normal regulated industrial hazardous waste, it is necessary to demonstrate that the vesicating properties of the chemical agents have been virtually eliminated.

B. Literature Search:

1. Literature Source(s) Searched:

The Current Contents® monthly computerized Life Sciences database is routinely searched for publications on biological effects of Chemical Surety Materiel (CSM) or dilutions. MEDLINE and TOXLINE are likewise routinely searched. On-line searches were conducted which included DTIC and FEDRIP to ascertain if previous studies (vesication) have been conducted on CAIS based on the process chemistries involved and utilization of the hairless guinea pig.

2. Date and Number of Search:

TOXLINE and MEDLINE were searched in July 95 for papers on sulfur mustard. DTIC and FEDRIP were searched in September 1995.

3. Key Words of Search: Key words used in searches included: sulfur mustard, hairless guinea pig, chemical agent identification sets (CAIS), CAIS wastestreams and/or components, and/or process chemicals, e.g. 1,3 dichloro-5,5 dimethylhydantoin (DCDMH), effects (vesication), sulfides, and sulfoxides.

4. Results of Searches: The hairless guinea pig model to measure microvesication as an indicator of dermal exposure to "blister agents" is the only well-accepted, published method to assess vesicating potential. No reports on vesication studies on CAIS/CAIS wastestreams in hairless guinea pigs were found.

III. OBJECTIVE/HYPOTHESIS:

The hypothesis of the study is that mean levels of skin irritation endpoints, including microvesication, at sites treated with neutralized CAIS compounds are statistically less than mean levels at sites treated with CAIS compound mixtures.

IV. MILITARY RELEVANCE:

The military relevance is discussed under background.

V. MATERIALS AND METHODS:

A. Experimental Design and General Procedures:

Phase I: Chemical Analyses and Validation of Methods

In the first phase of this task, analytical chemistry methods (e.g., gas chromatography-mass spectroscopy) proposed by ERDEC for determination of concentrations of HD, HN, and L in wastestreams will be evaluated. Modifications to these methods may be made based on the analytical equipment available in MREF laboratories and previous experience of chemists in the analysis of CSM. The need for extensive modifications is not anticipated. Each of the analytical methods, with any modifications, will be validated by evaluating the limit of detection, the limit of quantification, the linearity of response, and the precision, accuracy, and specificity. Analyses of the actual wastestreams for HD, HN and L concentrations will be accomplished following validation of the methods.

Phase II: Dosing Parameters for Agent(s) and Neutralization Solution

The objective of this phase is to assess the effects of dosing volume and/or exposure times for CAIS agents. Two sets of experiments are conducted in Phase II. In the first set, a small number of hairless guinea pigs are dermally dosed with the three agent solutions and with the neutralization solution using techniques for dosing chemical agents described in MREF SOP II-009 (enclosed). Using two animals at a time and a total of seven test sites per guinea pig, each animal is dosed percutaneously on both sides of the dorsal midline with low and high volumes of each CAIS agent mixture and with an optimal dosage of HD (approximately 1 μL) to demonstrate the effect of a known vesicant. On the first dosing day, agent is allowed to remain in contact with the skin for approximately 2 hr (based upon results obtained in earlier MREF studies with hairless guinea pigs and HD), and the skin then decontaminated with an approximately 0.5 percent sodium hypochlorite solution.* Approximately 24 hr following dosing, animals are sacrificed and skin samples from dosing sites are taken and fixed in formalin. Slides are prepared following tissue fixation, embedding, and sectioning, and evaluated for histopathology. Dosing volumes or times of exposure may be changed on subsequent days with additional animals to determine a volume and time of exposure for each CAIS agent mixture that results in consistent production of microvesication. Although the number of animals required to predict a dose volume and exposure time to consistently produce microvesication depends on the degree of microvesication observed in the first few animals, 4 to 12 animals are expected to be dosed. Once dosing volumes and times of exposure for each CAIS agent mixture are selected, the second set of experiments are conducted to verify consistent microvesication following administration of CAIS agent mixtures and to assess the extent of skin pathology following dosing of neutralization solution. For each CAIS agent mixture, dosing volume of neutralization solution is based upon the volume required to neutralize the volume of that agent mixture used to consistently create microvesication, but the maximum dosing volume at any site is limited to 100 µL. Five guinea pigs are tested to verify microvesication in at least 80 percent of sites dosed with each of the CAIS agent mixtures and to ensure lack of microvesication at sites dosed with neutralization solution. If, for any of the CAIS components, either skin microvesication is observed on sites administered neutralization solution or the incidence of microblisters at sites dosed with CAIS agents is less than 80 percent, experimental procedures will be modified and the experiment repeated. Up to 10 animals will be used.

If problems are encountered in meeting the objectives described above, experiments will stop, the Contracting Officer's Representative (COR) and the ERDEC Task Area Manager (TAM) apprised of the situation, and an alternative approach agreed upon (with concurrence on significant changes by the ERDEC LAURC Chair). If feasibility and appropriate dosing parameters can be determined, the next phase will start.

Phase III: Evaluation of Efficacy of Neutralization Process

The objective of this final phase is to demonstrate that the neutralization process substantially reduces the

* In studies performed at the MREF, a 0.5 percent sodium hypochlorite solution has proven to be an effective decontaminant for HD-exposed animal skin without causing obvious irritation.

vesicating properties of CAIS agent mixtures. Each animal is percutaneously dosed with CAIS agent mixtures and with volumes of wastestreams using parameters established in Phase II. Dosing volumes are selected to contain equivalent agent quantities or maximum volumes of 100 μL. Guinea pigs are sacrificed at approximately 24 hr after dosing and skin samples from dosing sites taken and prepared for histopathologic evaluation. It is anticipated that approximately 24 guinea pigs are required for this phase in order to demonstrate a statistically significant difference in the incidence of microvesication between sites treated with CAIS compounds and sites treated with neutralized CAIS compounds. If, after 12 animals have been dosed, a statistically significant ($p < 0.01$) difference is demonstrated, experimentation will cease at that time.

If problems are encountered in this phase, research will cease and the COR and TAM notified. Battelle and U.S. Army personnel will discuss problems and agree upon an alternative approach.

B. Laboratory Animals Required and Justification:

1. Non-animal Alternatives Considered:

   This task is necessary to determine if the vesicating properties of chemical agents have been virtually eliminated. This cannot be determined in other than a whole animal model.

2. Animal Model and Species Justification:

   The hairless guinea pig is an appropriate and useful model to assess HD-induced vesication of skin (Marlow, et al., 1990; Mershon, et al., 1990). The hairless guinea pig bioassay model will be used to determine the extent of vesication before and after neutralization of agents.

3. Laboratory Animals:

   a. Genus & Species: Cavia porcellus

   b. Strain/Stock: Crl:IAF(HA)-hrBR

   c. Source/Vendor: Charles River Lakeview (Newfield, NJ)

   d. Age: Guinea pigs will be approximately 3 to 4 weeks of age upon receipt.

   e. Weight: Guinea pigs will weigh approximately 200 to 350 g upon receipt.

f. Sex: Male guinea pigs will be used in this study.

g. Special Considerations: N/A

4. Total Number of Animals Required: 46 guinea pigs.

5. Refinement, Reduction, Replacement:

a. Refinement: Anesthetics will be used during exposure prior to decontamination.

b. Reduction: Experiments are conducted in a stage-wise fashion to limit the number of animals used to the minimum necessary to achieve statistically valid results. Procedures are stated for stopping experimentation if statistically significant results are obtained using fewer animals than expected to be necessary, or if problems are encountered. Results from previous studies at USAMRICD and at the MREF, and from previous phases of this study, will be used, as appropriate, to select doses and exposure times to limit the number of animals needed for this study to the minimum necessary to achieve statistically valid results.

c. Replacement: At the present time, vesication cannot be evaluated in other than a whole animal model.

C. Technical Methods:

1. Pain:

a. USDA (Form 18-3) Pain category:

Guinea pigs are anesthetized during the exposure period and it is believed that vesicating properties of the CSM will be virtually eliminated by neutralization and dilution. Positive control sites, i.e., sites dosed with CSM, have the potential to cause some pain, but animals are anesthetized during the exposure period. From past experience, guinea pigs do not appear to exhibit signs of pain following decontamination of vesicant agents. If signs of pain are exhibited following decontamination, buprenorphine at a dose of approximately 0.1-0.25 mg/kg sc can be given every 8-12 hr following consultation with a staff veterinarian or the study veterinarian.

(1) No Pain

(2) Alleviated Pain #46 100%

(3) Unalleviated Pain or Distress

b. Pain Alleviation:

(1) Anesthesia/Analgesia/Tranquilization:

Xylazine hydrochloride (approximately 6 mg/kg) and ketamine hydrochloride (approximately 35 mg/kg) will be given im to maintain anesthesia during exposure periods. Trained and experienced technicians will administer the anesthetics in the area of the hamstring muscles using a disposable tuberculin 1-mL syringe and a 23 to 25 ga needle.

(2) Paralytics: N/A

c. Alternatives to Painful Procedures:

(1) Source(s) Searched: TOXLINE, MEDLINE

(2) Date of Search: July 1995

(3) Key Words of Search: Mustard, Sulfur, Sulfur Mustard

(4) Results of Search: The hairless guinea pig model to measure microvesication as an indicator of dermal exposure to "blister agents" is the only well-accepted, published method to assess vesicating potential.

d. Painful Procedure Justification: N/A

2. Prolonged Restraint: Restraint lasting more than approximately 2 hr is not anticipated, and guinea pigs will be anesthetized during this period.

3. Surgery: No surgery will be accomplished.

4. Animal Manipulations:

Guinea pigs selected for study are anesthetized with approximately 6 mg/kg xylazine hydrochloride and approximately 35 mg/kg ketamine hydrochloride or other veterinarian-approved anesthetic agent(s). Following anesthetization, animals are positioned in sternal recumbency on restraint boards. A maximum of eight dosing sites are demarcated using an indelible-ink pen. Within a chemical fume hood, guinea pigs are dosed percutaneously with CAIS agent(s), neutralization solution, wastestream samples, and/or control compounds depending upon the phase of study. Guinea pigs will remain sedated/anesthetized during the time prior to decontamination with 0.5 percent sodium hypochlorite using additional doses of xylazine/ketamine (or other veterinarian-approved drug combination), as indicated. Following the decontamination of dosing sites, the animals are placed into individual polycarbonate cages within the hood. Use of Elizabethan collars may be necessary to prevent damage at dose sites. At approximately 24 hr following exposure, dose sites are evaluated for relative amounts of inflammation and pathology and approximation of size of any lesion. Guinea pigs are then euthanatized using deep inhalation anesthesia with halothane and death verified by opening the pleural cavity. Areas of skin at dosing sites are removed, placed in labelled cassettes, and put in a fixative solution. Tissue samples are processed and slides prepared by the Pathology Section of the Health Division or by MREF personnel and the slides are examined for histopathology and the presence or absence of microblisters by a qualified, experienced veterinary pathologist.

a. Injections: Anesthetics only.

b. Biosamples: No biological samples taken prior to necropsy.

c. Animal Identification: Ear tags or tattoos will be used to maintain positive identification.

d. Behavioral Studies: No behavioral studies will be done.

e. Other Procedures: N/A

5. Adjuvants: N/A

6. Study Endpoint: The study will end approximately 24 hr following dermal exposure to test compounds when the animal is sacrificed and skin samples taken.

7. Euthanasia: Euthanasia will be accomplished by trained and experienced laboratory animal technicians under the supervision of a veterinarian. Guinea pigs are sacrificed using deep inhalation anesthesia with halothane followed by creation of pneumothorax.

D. Veterinary Care:

1. Husbandry Considerations:

a. Study Room: Guinea pigs selected for study are anesthetized and positioned in sternal recumbency on restraint boards. Within a chemical fume hood, guinea pigs are dosed percutaneously with CAIS agent(s), neutralization solution, wastestream samples, and/or control compounds depending upon the phase of study. Guinea pigs will remain sedated/anesthetized using additional doses of xylazine/ketamine (or other approved drug combination), as indicated, prior to decontamination with 0.5 percent sodium hypochlorite. Following the decontamination of dosing sites, the animals are placed into individual polycarbonate cages within the hood and held there overnight. Water and feed will be available *ad libitum* overnight.

b. Special Husbandry Provisions: Hairless guinea pigs are held in isolation and observed for signs of clinical illness for at least 7 days prior to study initiation. Quarantine may be performed at Battelle's King Avenue Animal Resources Facility or at the MREF. All hairless guinea pigs are held at the MREF for at least 24 hr prior to study initiation. Hairless guinea pigs that are in apparent good physical condition after a minimum 7-day quarantine period are selected for study. An ear tag or tattoo is applied for positive identification of each hairless guinea pig. Before being used in experiments, hairless guinea pigs are housed individually in stainless steel or polycarbonate cages equipped with a watering system. Fluorescent lighting with light and dark cycles of 12 hr each per day is provided. Room temperature of holding rooms is maintained at approximately 64-79 degrees F. At least 90 percent of the twice daily recordings will fall within the specified range. Relative humidity will be maintained at approximately 40-70 percent. At least 90 percent of the twice daily readings will fall within the specified range. Purina Certified Guinea Pig Chow® pellets are available at all times prior to study initiation. No contaminants which would interfere or affect the results of the study are known to be present in the feed. Analyses of the feed are maintained. Drinking water is supplied from the city of Columbus public water system at Battelle's Animal Resources Facility at King Avenue and from private wells when animals are housed at the MREF, and is available *ad libitum* prior to study. No contaminants which would affect the results of the study are known to be present in either water supply. Water is analyzed annually for potability and contaminants.

2. Attending Veterinary Care: Guinea pigs will be held for only approximately 24 hr following dermal exposures

before being sacrificed. Veterinarians are on staff and available for any emergencies which might arise.

3. Enrichment Strategy: N/A

   a. Dogs: N/A

   b. Nonhuman Primates: N/A

E. Data Analysis: For chemistry validation data generated in Phase I, tables of means and standard deviations of response of each control standard are prepared to present both the inter- and intra- variability of the analytical method. Calibration performance characteristics for each run, such as slope and standard error of the slope, R^2 (measure of fit about the regression line), method detection limits, and quantitation limits are presented in a table format.

For Phase II, inflammation and histopathology data for each dose volume and exposure time of each CAIS agent(s) are summarized and tabulated.

For Phase III data, statistical hypothesis tests are conducted at the 5 percent significance level to determine whether or not the neutralization process reduced the vesicating properties of agents contained in CAIS. For each CAIS ampule, incidence of microblisters at sites treated with CAIS agent(s) are compared to those of contralateral sites treated with the neutralized wastestream. Although incidence of microblisters is the primary endpoint for evaluating the efficacy of each neutralization process, analyses are also conducted on signs of inflammation, lesion area, and other histopathology data. To accommodate the intra-animal correlation of multiple measurements made on the same animal, McNemar's test or conditional logistic regression analyses may be used to analyze quantal data. Analysis of variance (ANOVA) models that include random effects for animal are fitted to continuous data. If data are not approximately normal, ANOVA may be conducted on transformed data, or nonparametric or categorical methods of analysis may be performed.

To minimize animal usage, Phase III experiments are performed using a two-stage, group sequential hypothesis test. The first stage consists of the experimental results for twelve animals. For each neutralization process, an interim analysis is performed using the data from these twelve animals. If microblister incidence at sites treated with wastestream is statistically less than that of sites treated with CAIS agent(s), then the evaluation is considered complete for that process;

sites previously used for that process may be employed for evaluating the remaining processes. Otherwise, up to twelve additional animals are tested, and the efficacy of the neutralization processes reassessed. The significance levels of the interim and final analyses are carefully controlled to maintain an overall type 1 error rate of 0.05. This may be accomplished by selecting significance levels of 0.01 and 0.05 for the interim and final analyses, respectively. The two-stage hypothesis test is conducted using the microblister data only.

F. Investigator & Technician Qualifications/Training: The Study Director is an experienced research veterinarian and all animal technicians at the MREF are either AALAS certified as technicians or technologists or active in the AALAS training program. Records of their experience and training are available at the MREF.

VI. Biohazard/Safety: Surety, security, and safety procedures for the use of chemical agents are thoroughly outlined in facility plans, in personnel requirements for qualification to work with chemical surety materiel (CSM), and in standard operating procedures for storage and use of CSM.

VIII. ASSURANCES:

A. Animal Use: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein unless an amendment is specifically approved by the IACUC.

B. Duplication of Effort: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. Statistical Assurance: I assure that I have consulted with an experienced, well qualified statistician in the design and strategy of this study, and the minimum number of animals needed for scientific validity will be used.

D. Biohazard/Safety: I have taken safety into consideration in the design of this study and have made proper coordination in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" which the DoD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible, and conducting humane and lawful research.

Carl T Olson 11/29/95
(Study Director)

G. Painful Procedures: I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that will be relieved with the use of anesthetics. I have searched for alternatives to such procedures; however, I have determined that alternative procedures are not available to accomplish the objectives of the proposed experiment.

Carl T Olson 11/29/95
(Study Director)

IX. Enclosure:

Battelle MREF Protocol II-009

X. References:

Marlow, D.D., Mershon, M.M., Mitcheltree, L.W., Petrali, J.P., Jaax, G.P., Sulfur Mustard-Induced Skin Injury in Hairless Guinea Pigs, *J. Toxicol.-Cut. & Ocular Toxicol.*, 9(3), 179-192 (1990).

Mershon, M.M., Mitcheltree, L.W., Petrali, J.P., Braue, E.H., Wade, J.V., Hairless Guinea Pig Bioassay Model for Vesicant Vapor Exposures, *Fund. and Appl. Toxicol.*, 15, 622-630 (1990).

Evaluation of the Vesicating Properties of Neutralized
Chemical Agent Identification Set (CAIS) Components

Protocol Amendment No. 1

Change: Page 4, Section V. A. Experimental Design and General Procedures, Phase II: Dosing Parameters for Agent(s) and Neutralization Solution: Change the wording of the second sentence to read "In the first set, a small number of hairless guinea pigs are dermally dosed with the three agents using the techniques for dosing chemical agents described in MREF SOP II-009 (enclosed)." Change the wording of line 11 from "volumes of each CAIS agent mixture" to "volumes of each CAIS agent".

Page 5, Section V.A., Phase II: Change the wording on lines 9, 16, 19, 21, 23, and 27 from "agent mixture" to "agent" or "agent mixtures" to "agents".

Page 6, Section V.A., Phase III: Change the wording on lines 1 and 2 from "CAIS agent mixtures" to "CAIS agents". On lines 13 and 14, change "CAIS compounds" to "CAIS agents".

Page 11, Section V.E. Data Analysis, Change the second sentence of the third paragraph to read "Incidence of microblisters at sites treated with wastestreams is compared to that at sites treated with CAIS agent."

Reason for Change:

Instead of using actual ampules from CAIS kits, agent challenges will be prepared from HD, HN_1, and L stocks and will not be mixtures but individual agents. The neutralization solution will be dosed in the second set of experiments of Phase II, not the first as originally stated in the second sentence of the Phase II paragraph.

Change: Page 4, Section V.A. Experimental Design and General Procedures, Phase II: Dosing Parameters for Agent(s) and Neutralization Solution: After the existing second sentence, as modified, add the following: "Because agents on a charcoal matrix require extraction prior to dosing, and because the ratio of neutralization solution to agent on charcoal is 70:1, the combined volume of agent and neutralization solution for an agent dose that produces microblisters is greater than 100 μL. The maximum dosing volume is 100 μL. Therefore, it will not be possible to determine a volume of agent on a charcoal matrix that 1) produces microvesication and 2) can be neutralized and applied to a site. A sample of the wastestream from agents on charcoal will be tested for the ability to cause microvesication in Phase III, and will be chemically analyzed, but extracts of the agents on charcoal will not be tested in Phase II. Approximately 10 percent solutions of HD, HN_1, and L in chloroform, as well as neat HD, will be used to determine potential for creating microvesication. If, based on dosing volume limitations, one or two but not all three agents produce microvesication in the first set of Phase II experiments, the agent(s) producing microvesication in the lowest volume will be used to determine microblister formation following mixing of agent(s) with neutralization solution."

Reason for Change:

Because of limitations with dosing agents adsorbed on a charcoal matrix, it would be virtually impossible to administer a dose of agent which produces microblister formation combined with the neutralization solution in a total volume of less than 100 μL. Therefore, agents in chloroform solutions, as well as neat HD, will be used to challenge animals. Those agents that demonstrate the formation of microblisters at the lowest dose volumes will be used to determine if the neutralization process substantially reduces the potential for creating microblisters.

Impact on Study:

These changes are not anticipated to have an impact on the study since they involve dosing only one agent rather than a mixture, and a 10 percent concentration is probably the maximum that could reasonably be expected for any CAIS agent other than neat HD.

Approved By:

Carl T. Olson
Carl T. Olson, D.V.M., Ph.D.
Study Director

1/24/96
Date

Richard R. Stotts
Richard R. Stotts, D.V.M, Ph.D.
LTC, USA, VC
Contracting Officer's Representative

24 JAN 96
Date

Evaluation of the Vesicating Properties of Neutralized
Chemical Agent Identification Set (CAIS) Components

Protocol Amendment No. 2

Change: Page 1, Preface. Change to read:

"Study Performed by Battelle Memorial Institute's
Medical Research and Evaluation Facility
Building JM-3, West Jefferson, OH"

Reason for Change:

To clarify in the protocol the name and address of the testing facility where this study will be conducted, as required by 40 CFR Part 792.120 (3).

Change: Page 2, Sponsor. Add sponsor address so that this reads:

"Sponsor: Program Manager for Non-Stockpile Chemical Materiel (PMNSCM), USACMDA, Aberdeen Proving Ground, MD 21010-5425".

Reason for Change:

To add the sponsor's address to the protocol, as required by 40 CFR Part 792.120 (3).

Change: Page 2, I. Add, at the end of the paragraph, the following reference so that it reads:

"...Good Laboratory Practice (GLP) Standards (40 CFR Part 792)."

Reason for Change:

To add the specific reference for EPA GLP standards.

Change: Page 4, V.A., Phase I. Add at the end of the paragraph:

"The sponsor will provide wastestreams and HN. Composition of HD, L, and HN in CAIS also will be provided by the sponsor, and the components of the neutralization solution will be provided so that its preparation can be accomplished by MREF chemists."

Reason for Change:

To state compounds or documentation that will be supplied by the sponsor as required per 40 CFR Part 792.105 (a).

Change: Page 6, V.A., just prior to V.B., add:

"The first phase of this study will start after the receipt at the MREF of adequate quantities of HN and wastestream samples. Duration of the study is expected to be approximately 40 weeks to allow ample time for histopathologic evaluation of skin samples and analysis of results."

Reason for Change:

To add proposed experimental start and termination dates per 40 CFR Part 792.120 (4).

Change: Page 6, V.B.3.c., change to read:

"c. Source/Vendor: Charles River Laboratories."

Reason for Change:

The colonies of hairless guinea pigs needed for this study are raised at various locations and the source is not limited to the Lakeview colony.

Change: Page 14, IX. Change the word "protocol" to "SOP" so this reads:

"Battelle MREF SOP II-009."

Reason for Change:

This enclosure is an SOP, rather than a protocol.

Change: Page 14. Add section XI:

"XI. Records. Records to be maintained include, but are not limited to:

A. CSM accountability log and inventory
B. Chemical analyses and dose administration
C. Animal data
D. Clinical observations of lesions
E. Histopathologic evaluations of lesions
F. Decontamination, monitoring, and disposal records

Reason for Change:

This section was omitted from the original protocol and should be included per 40 CFR Part 792.120 (13).

Change: Page 14. Add section XII:

"XII. Reports.

A. A draft final report will be prepared within 30 work days after completion of the exposures and analyses of the data. The draft final report includes:

(1) Names of key study personnel
(2) Experimental design
(3) Test material description, analyses, preparation, and administration
(4) Clinical observations
(5) Histopathologic evaluations of skin samples
(6) Statistical analyses of data
(7) Discussions and conclusions.

B. Following receipt of sponsor comments on the draft final report, a final report will be prepared within 30 work days.

C. Interim results will be reported to the COR verbally during the course of the study, as possible.

Reason for Change:

This section was omitted from the original protocol and should be included per 40 CFR 792.185.

Impact on Study:

These changes are not anticipated to have any impact on the study, but are meant to clarify portions of or add to the protocol and are primarily administrative in nature.

Approved By:

Carl T. Olson — 2/6/96

Carl T. Olson, D.V.M., Ph.D.
Study Director

Date

Richard R. Stotts — 6 FEB 96

Richard R. Stotts, D.V.M., Ph.D.
LTC, USA, VC
Contracting Officer's Representative

Date

Evaluation of the Vesicating Properties of Neutralized
Chemical Agent Identification Set (CAIS) Components

Protocol Amendment No. 3

Change: Page 8, V.C.1.b.(1), Anesthesia/Analgesia/Tranquilization. Change first sentence of this section to read:

"Xylazine hydrochloride (approximately 13 mg/kg) and ketamine hydrochloride (approximately 87 mg/kg) will be given im to maintain anesthesia during exposure periods."

Page 8, V.C.4. Animal Manipulations. Change first sentence to read:

"Guinea pigs selected for study are anesthetized with approximately 13 mg/kg xylazine hydrochloride and approximately 87 mg/kg ketamine hydrochloride or other veterinarian-approved anesthetic agent(s)."

Reason for Change:

After the first day of study, it was evident that a xylazine dose of approximately 6 mg/kg combined with a ketamine hydrochloride dose of approximately 35 mg/kg was not sufficient to induce an adequate stage of anesthesia for a suitable period of time. Optimal doses of xylazine and ketamine to induce general anesthesia in guinea pigs for 60 min has been reported to be 13 and 87 mg/kg, respectively.[1]

Impact on Study:

This increase in dose of anesthetics should prevent the necessity of frequent booster doses of anesthetics and reduce any discomfort of the animals.

[1] Wixson, S.K., Rabbits and Rodents: Anesthesia and Analgesia, in *Research Animal Anesthesia, Analgesia and Surgery*, Smith, A.C. and Swindle, M.M., Eds., Scientists Center for Animal Welfare, Greenbelt, MD, September 1994.

Approved By:

Carl T. Olson, D.V.M., Ph.D.
Study Director

2/20/96
Date

Richard R. Stotts, D.V.M., Ph.D.
LTC, USA, VC
Contracting Officer's Representative

20 Feb 96
Date

Evaluation of the Vesicating Properties of Neutralized
Chemical Agent Identification Set (CAIS) Components

Protocol Amendment No. 4

Change: Page 6, V.A. Experimental Design and General Procedures: Phase III: Evaluation of Efficacy of Neutralization Process. Amend second sentence on this page, which reads "Dosing volumes are selected to contain equivalent agent quantities or maximum volumes of 100 μL." to read:

"Dosing volumes are selected to contain equivalent agent quantities, or are volumes of CAIS components and/or wastestreams selected by ERDEC, but do not exceed 100 μL. If microvesication is observed following dosing with one or more wastestream, major fractions of wastestream(s) may be used to dose animals in efforts to determine whether parent compound or degradation products are causing lesions."

Reason for Change:

The "blue" wastestream, that CAIS component containing neat HD neutralized with DCDMH, has been found to cause microvesication in HGPs at dosing volumes that contain agent-equivalent amounts. Although not one of the recommendations for further studies suggested by the MREF, personnel of the PMNSCM office requested the dosing of additional animals with the "blue" wastestream and with the 10 percent HD in $CHCl_3$ solution at equal volumes. Additional studies may be performed with major fractions of the wastestream in an effort to determine the component(s) that are creating the microvesication.

Impact on Study:

This change should not affect the overall objectives of the study. Dosing of fractions of the wastestream should help the identification of components creating the microvesication.

Approved By:

Carl T. Olson

Carl T. Olson, D.V.M., Ph.D.
Study Director

8/12/96
Date

Richard R. Stotts

Richard R. Stotts, D.V.M., Ph.D.
LTC, USA, VC
Contracting Officer's Representative

12 AUG 96
Date

Evaluation of the Vesicating Properties of Neutralized
Chemical Agent Identification Set (CAIS) Components

Protocol Amendment No.5

Change: Page 4, V.A. Phase I: Chemical Analyses and Validation of Methods

Change "Phase I: Chemical Analyses and Validation of Methods" to read "Phase I: Chemical Analyses and Evaluation of Methods."

Change sentence which reads "Each of the analytical methods, with any modifications, will be validated by evaluating the limit of detection, the limit of quantification, the linearity of response, and the precision, accuracy, and specificity." to "Each of the analytical methods, with any modifications, will be evaluated by determining the limit of detection, the limit of quantification, the linearity of response, and the precision, accuracy, and specificity."

Change the following sentence which reads "Analyses of the actual wastestreams for HD, HN and L concentrations will be accomplished following validation of the methods." to read "Analyses of the actual waste streams for HD, HN and L concentrations will be accomplished."

Page 11, V.E. Data Analysis

Change the first sentence from "For chemistry validation data generated in Phase I, tables of means and standard deviations of response of each control standard are prepared to present both the inter- and intra- variability of the analytical method." to read "For chemistry data generated in Phase I, tables of means and standard deviations of response of each control standard are prepared to present both the inter- and intra- variability of the analytical method".

Reason for Change:

Although the intent of Phase I of this study was to validate an analytical method for chemical vesicant agents in waste streams, no performance values were supplied to validate. Since this was the case, and because the provided method proved inadequate for the intended purpose and a modified method was developed under a separate contract, a precision and accuracy analysis, rather than a validation, of the method was performed by the chemistry section of the MREF.

Impact on Study:

This change should not affect the overall objectives of the study. The analytical method originally provided proved to be inadequate, and modifications were made to the method. Precision and accuracy measurements of the modified procedure were accomplished to define the capabilities of the modified analytical technique.

Approved By:

Carl T. Olson — 11/8/96

Carl T. Olson, D.V.M., Ph.D. — Date
Study Director

Richard R. Stotts — 13 Nov 96

Richard R. Stotts, D.V.M., Ph.D. — Date
LTC, USA, VC
Contracting Officer's Representative

Manual Number:

Battelle SOP MREF II-009-02
Page 1 of 9

STANDARD OPERATING PROCEDURE (SOP) FOR THE PERCUTANEOUS APPLICATION OF EITHER LIQUID OR VAPOR CHEMICAL SURETY MATERIEL OR OTHER IRRITANTS/VESICANTS ON SWINE AND GUINEA PIGS

Originated by: Thomas H. Snider — Date 02-Feb-1996
Thomas H. Snider, B.S.

Approved by: David Stitcher — Date 8 Feb 96
David L. Stitcher
~~MREF~~ Environment, Safety and Health Officer

Approved by: John B. Johnson — Date 8 Feb 96
John ~~B~~. Johnson D.V.M.
Co-Principal Investigator and Manager
Medical Research and Evaluation Facility

Reviewed and Registered by QAU: Elizabeth A. Cutter — Effective Date 2/12/96

Distribution List:

Quality Assurance Unit
SOP Manual(s)

Battelle
Health Division
505 King Avenue
Columbus, Ohio 43201-2693

I./II. Scope/Purpose

This standard operating procedure (SOP) describes all of the routine procedures for percutaneous application of sulfur mustard (HD), nitrogen mustard (HN), Lewisite (L), mustard Lewisite (HL) or other irritants/vesicants on swine or guinea pigs.

III. References

Battelle SOP MREF I-002, "Standard Operating Procedure (SOP) for the Storage, Dilution, and Transfer of GA, GB, GD, GF, TGD, VX, HD, HD/L, HN, and L When CSM Concentration/Quantity is Greater than Exempt Levels".

IV. Definitions

None

V. Procedures

A. Materials to be Used

CSM: HD, HL, HN, or L
Other irritants/vesicants

B. Hazards Involved

Hazards are listed in Battelle SOP MREF I-002.

C. Handling of CSM

The handling of CSM is conducted in accordance with Battelle SOP MREF I-002. The procedures used within this SOP that are described in Battelle SOP MREF I-002 include: entry and hood set-up at MREF, obtaining, equilibration, transfer, dilution, transport, and securing vesicants.

D. Equipment

The following is a list of equipment which may be needed in addition to that listed in Battelle SOP MREF I-002: animal clippers and blades, gauze pads, skin decontaminant kits or decontamination solutions, scales, MINICAMS® or other head-space samplers, felt-tip pen, ruler, approved anesthesia solutions, slings, timer, approved euthanasia solutions, notebooks, blunt-tipped needles, microliter syringes, vapor cap assemblies, occluding material, o-rings, cyanoacrylate adhesive, Elizabethan collars, caging, underpads, tissue casettes, formalin solution, sharps container, warming pads/pumps, plastic bags, labels, scalpel handles and blades, volumetric pipettes, gas chromatography vials and caps, adsorbent material, large plastic jars with lids, dosing grid template, observation table, lab chair, calculator, disposable syringes, positive-displacement micropipettors and tips, trypan blue, tape, scissors, forceps, tongue depressors, bell jar, plastic-backed paper, brown kraft paper, holding boards, distilled water, cardboard pieces, surgical tape, and vet-wrap.

E. Preparation of Animals

Technicians working in the preparation room are, at a minimum, required to wear scrub suits, latex/nitrile gloves, and protective eyewear. If required by protocol, animals are anesthetized. Following initial anesthesia via injection or inhalation, booster anesthetic agents may be administered. A catheter may be inserted into a marginal ear vein and additional anesthetic provided intravenously. Booster doses of anesthetic may be administered to some species by intranasal drip or by inhalation. According to the procedures outlined in the specific protocol, the back of the animal may be cleaned prior to dosing. Dosing areas are marked as specified in the protocol. If required, Viton®, Teflon®, or rubber O-rings may be affixed to the back at dosing sites. If the protocol specifies removal of the animal from the hood on the day of dosing, double-sided tape is placed around the dosing area for affixing an air sampling container for performing proof-of-decontamination (POD). An animal may be secured to a restraint board (guinea pig) or in a sling (swine). Swine in slings will have their legs restrained to the posts of the stand. Animals are maintained in a fully anesthetized state while on restraint boards or in slings. Additional anesthetic agent will be readily available.

F. Preparation of Dosing Syringes

Syringes and blunt-tipped needles used for dosing will be chosen on the basis of the agent and the volume being applied. In general, the following guidelines are used.

Needles	Syringes
HD, HN - Stainless-steel	< 0.1 μL - Micrometer syringe device
L - Platinum, iridium, or gold-plated	0.1-1.0 μL - syringe, e.g., Hamilton 7001

HL - Platinum, iridium, or gold-plated	1-10 μL - syringe, e.g., Hamilton 701
Others - As appropriate	10-100 μL - syringe, e.g., Hamilton 1710 LT
	10-500 μL - syringe, e.g., Hamilton 1750 LT
	100-1,000 μL - syringe, e.g., Hamilton 1001 LT

The irritant/vesicant is drawn into the syringe following the procedure outlined in Battelle SOP MREF I-002. Once the syringe is properly filled, the syringe is placed on an adsorbent pad if more syringes are to be filled, or the content is expressed onto the designated dose site on the animal. The empty syringe is then placed on the adsorbent pad or reloaded for additional applications. After the appropriate number of syringes are filled, or the applications are made, a new lid is placed on the primary container.

G. Preparation of Dosing Micropipettors

A positive-displacement micropipettor may be used for the delivery of multiple aliquots. The doser should be aware that the potential exists for movement of the agent up the micropipettor neck and onto the surfaces of the mechanical parts inside the micropipettor. The doser should assume that such contamination has occurred, and following dosing, suspend the micropipettor on the rim of an Erlynmeyer flask containing distilled water to a level such that the plunger wire is submerged. After an approximately 24-hr period, the micropipettor may be dried and placed into a plastic bag and the bag sealed.

After each storage period and before use, the outer surface of the pipettor is examined for possible contamination. If there is any liquid on the exterior of the micropipettor, the pipettor is discarded by submersion in decontamination solution, and the doser removes and decontaminates his butyl gloves per SOP MREF I-002. If the micropipettor exterior appears uncontaminated, the micropipettor may be used. The doser examines the micropipettor to assure the proper volume setting.

The doser places the micropipettor on brown kraft paper or other disposable adsorbent surface, and picks up a plastic-backed wipe with one hand and a tip designed for that micropipettor in the other hand. The tip is surrounded by the wipe and held in one hand, the micropipettor is picked up in the other hand, and the tip put in place. The wipe is placed into decontamination solution. The tip is secured on the micropipettor. The tip diameter must be small enough to allow entry into the primary container of the CSM or irritant.

The micropipettor is placed on brown kraft paper or other disposable adsorbent surface. The primary CSM or irritant container is uncapped per SOP MREF I-002. The micropipettor is picked up, and the tip inserted through the container opening until the tip point is just submerged. The micropipettor is never inserted to a position past the top of the disposable tip, which could cause contact of the micropipettor with the primary container's inside surface. The container is not appropriate for dosing with a micropipettor if the liquid level cannot be contacted without inserting the pipettor into the container.

The plunger is slowly allowed to retract, and agent or irritant is pulled into the tip. The viscosity of the material may require a pause to allow equilibration of pressure in the tip. The doser removes his thumb from the micropipettor plunger. The tip is withdrawn and wiped with a plastic-backed adsorbent paper which is then discarded into a beaker of decontamination solution. The doser moves the micropipettor to a position over the dosing site, and the plunger is carefully depressed to extrude the dose volume. If the used tip appears to be empty after dose delivery, it may be reused to perform multiple volume transfers. After the final use, decontamination solution is drawn from a beaker into the tip and dispensed into the waste beaker three times. The tip is removed and placed into a sharps container with decontamination solution.

H. Percutaneous Application of Liquid Irritant to Dose Sites

An individual wearing clean gloves will monitor the animal prior to dosing to ensure proper depth of anesthesia. If gas anesthesia is used, the anesthesia line will be secured prior to dosing. The volume of irritant applied to a particular dose site is protocol specific. Doses are applied to the center of each dose site using a syringe or pipettor with a blunt-tipped, positive-displacement needle. The tip of the needle may be touched to the application site to ensure transfer of material to the site from the syringe tip. Dermal applications requiring volumes larger than 5 μL may be made in incremental applications to the prescribed sites. Doses may also be applied as a streak. After dosing and any protocol-specified treatment and/or decontamination procedures, if applicable, are completed, experimental animals will be shifted within the hood system by the operations assistant. If possible, all animals will be positioned with their heads oriented toward the front of the hood. The animals will remain in this position until the end of the observation period. If the protocol requires holding guinea pigs within the hood system overnight, animals will be removed from their restraint and housed in appropriate caging with access to water. Feed may be made available if approved by protocol, the study director, or a staff veterinarian.

I. Percutaneous Exposure by Vapor Cap Assembly

An assistant wearing clean gloves will monitor the animal to ensure proper depth of anesthesia. If gas anesthesia is used, the anesthesia line is secured prior to dosing. When the irritant/vesicant is administered as a vapor to an occluded dose site, it is dosed into a vapor cap assembly. The vapor cap assembly consists of a plastic cap with a smooth, flat rim of approximately 1 mm thickness, and a filter paper wafer placed inside the cap top. Carpet tape with adhesive on both sides is covered with release paper on both sides, appropriately sized openings for dosing made, and the tape cut into a size appropriate for mounting on the animal's back. Dose sites are marked on the animal's back as specified by protocol. If prescribed by protocol, a topical skin protectant (TSP) is applied to the dose site in an area slightly larger than the dosing perforation cut in the tape. The tape is applied to each dose site by removing the release paper from one side and firmly pressing that side onto the skin at the dose site.

Syringes or pipettors are selected based on the dose volume to be delivered and filled as described in Section F or G. The vapor cap assembly is placed upside down in a shallow well, approximately the same diameter as the cap and with a depth of approximately half the height of the cap, drilled into a holding block . At the time prescribed by protocol, the plastic film is removed from the top of the tape which is adhered to the skin at the dose site. The irritant is dispensed directly onto the paper wafer in the cap, taking care not to touch the needle or micropipettor tip to the rim of the cap. After the dose is delivered, the needle tip or micropipettor tip may be touched to the paper wafer, and the delivery device placed on a disposable adsorbent surface within the hood. The vapor cap assembly is then lifted out of the well with forceps, inverted, and placed onto a protocol-specified surface. After a designated time interval, each cap is transferred by sliding it onto a vapor cap transfer apparatus. The cap is then centered over the opening in the tape at a dose site and the cap top is lightly pressed with forceps to ensure a seal between the rim of the cap and the carpet tape. After receiving a particular treatment, animals are shifted within the hood system by the operations assistant. If possible, all animals are positioned so their heads are oriented toward the front of the hood. The animals remain in this position until the end of the observation period.

J. Decontamination of Irritant/Vesicant on Skin with a Test Decontaminant

Protocols may require the evaluation of test decontaminants on percutaneously dosed animals. The following describes the standard test decontamination procedure. Additional decontamination operations may be outlined within the protocol.

The dosing assistant or operations assistant applies a measured quantity of the test decontaminant to a swab (adsorbent padding, gauze, or fibrous pad secured to a tongue depressor(s)) or a decontamination mitt. At the scheduled time, the dosing assistant initiates decontamination by physically stroking or tapping, as specified by study protocol, the dosed area with a brisk action perpendicular to the animal's spine. For solid or powdered test decontaminants, a piece of cardboard may be held behind the dosing area to minimize sprread to other sites. After use, all swabs or mitts and other materials utilized for decontamination are deposited into a waste decontamination beaker. Quantity of test decontaminant to be applied, length of exposure before decontamination, and length of the decontamination process may be specified in the protocol.

K. Decontamination of Irritant/Vesicant following Vapor and/or Liquid Exposure

After a vapor exposure period, the vapor cap assembly is removed intact with the tape by gently pulling up on the folded tape tab with forceps. The vapor cap assembly and tape are placed into a bucket of the appropriate decontamination solution (e.g., 5 percent sodium hypochlorite ($NaOCl$)).

As specified by protocol and as directed by the sponsor, the dosed skin site may or may not be decontaminated following exposure. However, decontamination is necessary if the animal is to be removed from the hood.

In studies not involving TSPs, a decontamination assembly, consisting of either a 4 x 4 inch gauze pad or an adsorbent pad wrapped and taped around a wooden tongue depressor, is used to decontaminate the skin. A dry gauze pad is laid over the dose site for the protocol-specified length of time. An adsorbent pad assembly is moistened with a 0.5 percent $NaOCl$ solution or other specified decontamination solution, and used to neutralize or remove any residual irritant from the dose site. The dose site is then rinsed using adsorbent assemblies moistened with distilled water. Once used, the assemblies are placed into decontamination solution.

If the protocol is for screening TSPs, a dry adsorbent may be used to wipe the TSP from the skin. The dose site is rinsed or treated as specified in the protocol. Any adsorbents used are placed into decontamination solution.

L. Euthanasia, Decontamination and Removal of Animals

(1) Animals remaining in the hood overnight:

If specified by protocol, at the end of the observation period, the animals are anesthetized and an aqueous solution of trypan blue administered to aid in visualizing lesions. Upon completion of the experiment, all animals are euthanatized, either by administering an injection of a lethal dose of an approved euthanasia solution, or as specified by protocol. Tissues and/or skin samples may be taken if specified by protocol. The exposure site(s) and any possibly contaminated sites of all animals are treated with a forceps-held gauze pad moistened with 5 percent NaOCl or other specified decontamination solution. Following the decontamination procedure, all gauze pads are discarded into a decontamination beaker.

Euthanatized and decontaminated animals are placed into a plastic bag. The plastic bag is brought to the hood face by a fully garbed technician. Outside the hood, a second technician awaits with a second or multiple layers of plastic bags, as specified. As the technician stationed outside the hood brings the bag(s) to the hood face, the technician working within the hood carefully places the bagged animals from inside the hood into the bag(s). The opening of each bag is twisted, folded, and taped. Labelled bags are brought to the POD area for analysis. All carcasses are to be incinerated after POD.

(2) Animals removed from the hood on the day of dosing:

A static air space over the dosing sites is monitored using air samplers. To achieve a static air space, a fitted flexible polyethylene container is secured to the animal's back over the dosing sites. The tightness of the seal is examined prior to initiation of sampling. (Note: The volume of the air space should be approximately 1 liter.) After the proper equilibration period, the cap from the sampling port is removed and a MINICAMS® sampler probe or other air sampling device is inserted into the static airspace. Animals cannot be removed from the hood until irritant/vesicant vapors are below established airborne exposure limits. When POD is obtained, clean gloves are donned, and the anesthetized animal is removed from the hood and placed into a holding cage. When the appropriate endpoint measurements have been made, the animal is euthanatized with an approved solution and, if applicable, skin samples are collected and placed into a formalin solution or other specified fixative. Animal remains are incinerated following the collection of skin samples.

M. Decontamination, Emergency Procedures, and First Aid Procedures

These procedures are described in Battelle SOP MREF I-002. All staff conducting these procedures are required to read, sign, and be familiar with Battelle SOP MREF I-002.

N. Quality Control

There are no procedure-specific quality control measures required for this SOP. When required, they are stated in the study protocol under which this SOP is performed.

DEVIATION REPORT

G155538A

Evaluation of the Vesicating Properties of Neutralized
Chemical Agent Identification Set (CAIS) Components

Type of Deviation: SOP

During the compiling and quality control review of data generated during the conduct of the study, the Task Leader found that there were certain events that were performed not totally in accordance with an SOP. These events are listed below.

Date of Deviation: January 21, 1996.

Nature of Deviation: The relative humidity in Room 41 was recorded at 35% on this Sunday and there was no documentation of it being monitored, per MREF IV-001.

Cause of Deviation: Technician either did not realize the need to document or forgot to document additional readings which the SOP says will be taken when readings are outside the acceptable SOP range.

Impact on Study: None.

Corrective Action: Technicians working weekends will be reminded that "monitoring" out of range readings should be interpreted to mean that documentation of subsequent readings should be recorded during the time technicians are working in the facility.

Scott Bryant
2/28/97

Date of Deviation: March 5, 1996.

Nature of Deviation: The temperature and relative humidity conditions in Room 6, where the animals were prepared for dosing on this day, were inadvertently not recorded per MREF IV-001.

Cause of Deviation: Technician forgot to record these conditions.

Impact on Study: None.

Corrective Action: Technician was reminded to record these conditions per SOP. Also, when reviewing these records in December 1996 and January 1997, it should be noted that the SOP was revised in November 1996 to eliminate taking temperatures and humidities in non-animal rooms as this is not a necessary facility requirement. Because this technician no longer works at the MREF, I am signing as his supervisor

Dates of Deviation: August 16, 1996 through August 26, 1996.

Nature of Deviation: The check for vermin in Room 17 was not documented per MREF VII-002.

Cause of Deviation: Technician forgot to document this check.

Impact on Study: None.

Corrective Action: Technician was reminded to document this check weekly. TLB

Date of Deviation: August 29, 1996.

Nature of Deviation: Room 6 was unavailable for animal preparation procedures so animals were prepared in another room which did not have a thermometer and hygrometer present per MREF IV-001.

Cause of Deviation: Staff member forgot to monitor these conditions.

Impact on Study: None.

Corrective Action: Staff member was reminded to record these conditions per SOP. Also, when reviewing these records in December 1996 and January 1997, it should be noted that the SOP was revised in November 1996 to eliminate taking temperatures and humidities in non-animal rooms as this is not a necessary facility requirement. PCK

Prepared by:

D. Marie Moore

D. Marie Moore, Task Leader

2-13-97

Date

Approved by:

Carl T. Olson

Carl T. Olson, Study Director

2/21/97

Date

Standard Operating Procedure (SOP) Deviation Report

Study Number: G1555-38A

Study Title: Evaluation of the Vesicating Properties of
Neutralized Chemical Agent Identification Set (CAIS) Components

SOP Deviation to MREF III-002: SOP for the Measurement of Chemical Surety Materiel in Dilute Solutions of GA, GB, GD, TGD, GF, HN, HD/L, HD, L, and VX

Dates of Deviation: 2/20/96, 2/22/96, 2/27/96, 3/5/96.

Nature of Deviation: This SOP was revised in version -02, which became effective on March 6, 1996, to include the analysis of HN-1. Some HN-1 analyses were performed in February and early March on GLP study G1555-38A before version -02 was signed.

Cause of Deviation: Lag time in getting the new version approved and distributed.

Impact of Deviation on the Study: None. The methods used for HN-1 analysis are the same as those for HD, as stated in this SOP.

Corrective Action: None necessary.

Approved By: [signature] Date: 3/27/96
Study Director

Approved By: [signature] Date: 27 MAR 96
Study Sponsor

G1555-
Study # 38A

ANIMAL WEIGHT SHEET

Project Number: G1555-38A

Species: H.G. Pig

C... LE
2-2-96

Animal I.D.	Date: 2-2-96 Weight (~~kg~~)
301	304.6
302	327.9
303	290.1
305	300.2
306	304.3
307	292.9
309	305.8
Tech. Init. DMM	

Note: These animals were assigned to a specific study until several days after receipt. Therefore, initial wt were not taken until 2-2-96. In order to help track their wt gain, they were again weighed on 2-9-96 and 2-16-96.

Robyn C Kiser
3-27-96

Carl T Olson 10/25/9

N/A = Not applicable

Balance Calibration: 100.0g 500.0g / 100.0g 500.0g

Reviewed by: Robyn C Kiser

Balance I.D.: 183284

Date: 2-2-96

Weight I.D.: C11365

Calibrated by: DMM

Date: 2-2-96

Form No. MREF-WEIGHT.SHT-02.1
Appendix A

G1555-38A

CD® CD-1® CHARLES RIVER LABORATORIES CD® CD®

CUSTOMER MASTER LABEL

8 IAF/HA-HO G. PIGS MALE
250-300 GMS 23-29 DAYS
PORTAGE AREA: P02

ACCOUNT #: 01825-01-041 PO #:115941
CONTROL # 1159454 SHIPMENT REF # 29612266
SHIPDATE:02/26/1996 DELIVERY DATE:02/27/1996

TOTAL CRATES: 1 TRANSGEL PER CRATE: 1

008 01/31/1996

BATTELLE MEMORIAL INSTITUTE

INVESTIGATOR NAME: FRANCES REID
SPECIAL REQUIREMENTS

CFW® MINIPIG-YU® This sale is made on the terms on our current price list and no other. Acceptance of this delivery constitutes your acceptance of those terms. MICROPIG® MINIPIG-HA®

8 hairless guinea pigs arrived at MREF at approx. 12:30pm on 2-27-96. These were not originally ordered for Task 95-38, however, several days after their arrival, they were assigned for Task 95-38 use.

Robyn C Kiser

G1555-38A

Project No.: G1555-2301 Test Agent: —

Animal Species: Hairless Guinea Pig Sex: M

Date: 2-27-96 Initials: GW.

Animal No.	Weight (g)	Male
499	261.3	
498	271.6	
497	250.0	
496	245.7	
494	275.8	
493	272.5	
492	275.0	
491	262.0	

These 8 hairless guinea pigs arrived at the MREF on 2-27-96. On 3-1-96 it was decided that they would be assigned for use on Task 95-38. Weights were taken on the day of receipt, however, a record of balance calibration checks, balance used, etc was not documented at that time since these animals were originally ordered for a non-GLP study. These animals were from the same source, all male, and received within the required weight range for Task 95-38 (G1555-38A).

Robyn C Kiser
3-1-96

Carl T Olson 10/30/96

Date: August 6, 1996
To: G15538A Study File
From: Marie Moore, Task Leader
Subject: Under-Weight Hairless Guinea Pigs

For Protocol 109, during the first week of August, Charles River Laboratories was requested to send eight male Hairless Guinea Pigs weighing approximately 200-225 g on arrival so that the animals could be held for studies to be run later in the month and still not be excessively heavy. The protocol actually requires animals to weight approximately 200-350 g on arrival. On August 6, 1996, eight Hairless Guinea Pigs were received with three of the animals weighing under 200 g upon arrival. The study director is aware of this and does not anticipate any problem or adverse impact on the study.

Carl T Olson 10/25/96

APPENDIX B

Analytical Methodology

Task 95-38 Analytical Method Verification
G155538A

INTRODUCTION

This report summarizes the results of precision and accuracy testing of a gas chromatographic method with mass spectrometric detection (GC/MS) used for the determination of bis(2-chloroethyl)sulfide (HD), bis(2-chloroethyl)ethylamine (HN-1), and dichloro(2-chlorovinyl)arsine (L) in solutions neutralized by 1,3-dichloro-5,5-dimethylhydantoin (DCDMH) oxidation in organic solvent (Rapid Response System [RRS] neutralization). The analytical method was classified as "Interim" by the developer, but approved for use at Battelle's Medical Research and Evaluation Facility (MREF) by Edgewood Research, Development and Engineering Center (ERDEC) personnel. RRS neutralization samples were prepared by ERDEC personnel using the neutralization process for the method verification. These samples are referred to as waste streams or spent neutralization solutions in this report.

The RRS was designed by personnel from the office of the Program Manager for Non-Stockpile Chemical Materiel (PMNSCM) to neutralize vesicating compounds from field training kits (Chemical Agent Identification Sets) containing HD, HN-1 and L. These chemical warfare (CW) materiel are in organic solution (HD,HN,L), neat (HD), or adsorbed on granulated charcoal (HD,HN,L), and contained in sealed glass ampules. The analysis method designed requires a GC/MS system capable of splitless injection into a fused silica capillary column, analyte ionization by electron impact (EI), ion detection in the selected ion monitoring (SIM) mode, and a dedicated PC-based MS data processing capability.

METHODS

Method Summary

Method performance was determined through recovery from spent neutralization solution of agents added at known levels. Since the waste streams were expected to have residual oxidizing power, a direct spike into a sample waste stream, as received, would not be meaningful. Therefore, both the residual oxidant and the strong acidity (HCl) anticipated in the waste stream were quenched prior to the spike. Water soluble impurities were removed from the waste stream by partitioning to an aqueous buffer. In addition, L was derivatized prior to analysis by reaction with 1,3-propanedithiol (PDT) to form the arsenic disulfide derivative, 2-(2-chlorovinyl)-2-arsa-1,3-dithiocyclohexane [Cl-CH=CH-As(-$SCH_2CH_2CH_2S$-)], or L-PDT. The L-PDT derivative is referred to as L-Der in this report. An internal standard (IS), 1,2,4,5-tetrachlorobenzene, was used in agent quantitation and for GC retention time comparisons. Samples were analyzed without concentration of the extract, and quantitation was accomplished using calibration standards prepared in the extraction solvent mixture. Agent detection was assured for each waste stream sample through the recovery of an over-spike of the corresponding CW materiel within the sample matrix. Inadequate recovery from spiked samples was presumed to be a result of sample matrix effects. These matrix effects were overcome by diluting and reanalyzing the sample.

The analytical method was developed by Dr. Samuel V. Lucas of Battelle's National Security Division, with the assistance of MREF chemists, under another task, and a copy of the method is attached to this appendixas Attachment A. The method was classified as "Interim" by Dr. Lucas, but MREF project staff were directed by ERDEC personnel to use the method.

Instrumentation

A Hewlett Packard Model 5970B Mass Selective Detector (MSD) was used for method verification and for subsequent waste stream analyses. The HP 5970B MSD is a stand-alone detector used in the MREF laboratory with an HP 5890A GC and the HP G1034C MS ChemStation for automating the analyses. The instrument conditions are presented in Attachment A.

Method Verification

There were three phases to the method verification.

Phase I. GC/MS Instrumental Performance with Calibration Standards

Calibration standards were prepared as mixtures containing approximately equal weights (μg/mL) of HD, HN-1, and L-Der in the extraction solvent mixture at each analysis level. Triplicate preparations of each of five standards were prepared along with ten replicate dilutions of a 3 ppm standard. Initially, six standards were used but results indicated that the L-Der could not always be detected at or below 1 ppm so the 0.3 ppm standard was dropped from the data set. All three sets of standards and the ten separate dilutions of the 3 ppm standard were analyzed as a single sequence. The ten replicate injections from a single 3 ppm standard were included in the sequence to determine the instrument precision.

Phase II. Method Performance with Process Blanks

Process Blank is the term used in the method to indicate "control samples" taken through the entire sample work-up procedure. Seven separate spiked Process Blank extracts from each spike level - 2.5, 10, and 25 ppm (5 ppm was used for L instead of the 2.5 ppm due to the weak signal at 2.5 ppm) - were prepared and analyzed in a single sequence along with a set of five calibration standards and a Process Blank without agent.

Phase III. Method Performance with RRS Matrix (Waste Stream) Samples.

Three waste streams, identified below, were analyzed using the method. These samples were analyzed both as received (no agent spike) and in duplicate at each spike level - 2.5, 10, and 25 ppm (5 ppm for L instead of 2.5 ppm). They were analyzed in a single sequence with a set of five calibration standards, and 0 and 25 ppm spiked Process Blank samples on three different days.

The three waste streams used in Phase III were identified as:

#4 Blue Process Fresh 5/96 lot # 96-0037-049
#5 Red Process Fresh 5/96 lot # 96-0037-047
#3 Charcoal Process 5/96 lot # 96-0037-014 L, -018 HN-1, -023 HD

These waste streams are referred to as Blue, Red and Charcoal in this report.

RESULTS

Each compound analyzed by MS has a characteristic fragmentation pattern, and this pattern along with the analyte's retention time provided a high degree of selectivity to the GC/MS technique. SIM data were acquired for the six ions specified in the method for each agent, and for the IS. Quantitation was achieved by using the target ion for each agent and the IS as specified in the method. The ratio of the peak area for the target ion of the analyte to the peak area of the target ion of the IS was used in the regression analysis. These quantities are referred to as target area and response ratio in the tables and figures of this report. The ChemStation analysis routine not only integrated the target ion peak area in the chromatogram, but also evaluated the relative response among three additional ions. In the ChemStation report, the retention time and target area were provided as well as the relative response of the three secondary, qualifying ions and an indication of whether the specified relative response criteria for the qualifying ions being investigated were met. On a few occasions when the analyte was absent (Process Blank or Waste Stream with no spike), the ChemStation integrator would report small target area values. In these cases a small shift in retention time was observed and the ChemStation report would indicate that the qualifications were not satisfied. Consequently, a value of zero was assigned as the target area by the analyst. The three dimensional data of time *vs* detector signal *vs* mass to charge ratio (m/e) add a significant degree of specificity to the method. The overall method specificity, however, was not evaluated in this study.

The SIM results for Phase I of the method verification are summarized in Table 1 of Attachment B (pages B-20 to B-22). The calibration curves for the agents are shown in Figures 1 to 3 on pages B-23 to B-25, and regression analysis results for the standards are presented in Table 2 on page B-26. Descriptive statistics for the three independent sets of calibration standards and the ten 3 ppm standards are summarized in Tables 3 and 4 on pages B-27 and B-28.

The SIM results for Phase II are summarized in Table 5 on pages B-29 to B-31. Regression analysis results for the standards analyzed with the Process Blank extractions are given in Table 6 on page B-32, and descriptive statistics for Phase II are summarized in Table 7 on page B-33

The SIM results for Phase III are summarized in Table 8 on pages B-34 to B-42. Regression analysis results for the standards analyzed with the waste stream extractions are given in Table 9 on page B-43, and descriptive statistics for Phase III are summarized in Table 10 on pages B-44 to B-49. A summary of the descriptive statistics for the waste stream samples over the three days of analyses are presented in Table 11 on pages B-50 to B-54. The percent recovery for each agent from the waste stream samples are presented graphically in Figures 4 to 6 on pages B-53 to B-55.

DISCUSSION

Phase I. GC/MS Instrumental Performance with Calibration Standards

Calibration fits were linear for HD and HN-1 and simple linear regression models were employed. A small but statistically significant quadratic effect was present for L-Der (Figures 1 to 3, and Tables 2 and 4). Therefore, all of the results reported for L-Der using calibration standards are based on a quadratic model. The regression models for all three sets of calibration standards were significant at the $p < 0.001$ level, and r-squared values were all greater than 0.99. Statistical tests performed on the three independent sets of calibration standards demonstrated no significant differences between batches with respect to the regression fits of each analyte.

It was determined from preliminary data that reproducible results for the L-Der at 1 ppm were not practical. Therefore, the 3 ppm calibration standard was replicated to estimate the limit of detection and the limit of quantitation. The instrument limits of detection and quantitation were estimated with and without sample preparation. These values are reported in Table 3. Ten separate injections from a 3 ppm calibration standard along with a single injection from ten separate preparations of the 3 ppm calibration standard were used. The detection limit was computed as the concentration which corresponds to a signal that is three times the value of the standard deviation found for the lowest calibration standard. The quantitation limit is defined in a similar way using ten times the standard deviation. The detection limits found for the instrument were 0.4, 0.7, and 2 ppm for HN-1, HD, and L-Der, respectively. The quantitation limits for the instrument were approximately 1, 2, and 3 ppm for HN-1, HD, and L-Der, respectively (see Table 3).

Precision is evaluated as the relative standard deviation, which is reported as percent and is referred to as the coefficient of variation (C.V.) in the results tables. The precision of the instrument, as based on the analysis of calibration standards, is about $\pm$ 5 percent for all of the analytes, and sample preparation adds about another $\pm$ 1 percent (see C.V. in Table 3).

In Table 4, the relative standard deviation is given at each of the five concentration levels for the three independent sets of calibration standards. The value is 5 percent or less for all concentration levels for HD and HN-1. The relative standard deviation for L-Der increases with decreasing concentration so that at 1 ppm, the relative standard deviation is approximately 18 percent. Both the 1 and 2.5 ppm L-Der standards are less than the quantitation limit. Likewise, the error associated with the back-calculated concentration of the standards shown in

Table 4 are all well below 10 percent except for the two lower L-Der standards. The errors in the 0.8 and 8 ppm back-calculated concentrations for L-Der are both greater than 10 percent.

Phase II. Method Performance with Process Blanks

In Phase II, seven separate spiked Process Blank samples were extracted and analyzed at each spike level - 2.5, 10, and 25 ppm (due to sensitivity issues, a 5 ppm spike was used for L instead of 2.5 ppm). The relative standard deviations (C.V. in Table 7) were all less than 10 percent (range of 1.2 to 9.3 percent). At 25 ppm, all of the relative standard deviation values were less than 3 percent, indicating that the reproducibility of this multiple-step extraction process is good. The accuracy is expressed in percent recovery and is best for the 25 ppm: 105 percent for HD, 90 percent for HN-1, and 123 percent for L (Table 7). Since the percent recovery for 10 ppm HN-1 is much lower than that for 2.5 and 25 ppm HN-1, it was assumed that an error in preparing the solution used for this dilution had occurred. Each of the three spike levels were achieved by preparing three separate solutions and spiking each sample with 100 μL of the appropriate stock (both HD and HN-1 were mixed in a single solution at each level and L was prepared separately). Over the complete spiking range, the HN-1 concentration values (excluding the 10 ppm spiked sample discussed above) were underestimated by 10 to 15 percent. Except for the 25 ppm HD sample, which is 5 percent high, HD and L spiked concentration values were overestimated by 12 to 35 percent.

Phase III. Method Performance with RRS Matrix (Waste Stream) Samples

Low levels of HD or L were detected in all of the waste stream samples. The average concentration values over the three days were 12 ppm of HD in the Blue, 25 ppm of L in the Red, and 20 ppm of L in the Charcoal (Table 11).

The absolute recovery for the method was determined by spiking the waste stream samples. The three spike levels used in Phase II were used with each waste stream, and a 25 ppm spiked Process Blank sample was prepared. All of these samples were extracted with iso-octane for GC/MS analysis as outlined in the method. Calibration standards were analyzed sequentially with each set of extraction samples, and the regression analysis results for the calibration standards were used to determine the concentrations of HD, HN-1, and L in the initial 1-mL sample. Although L-Der is analyzed, the analysis reporting procedure calculates the concentration of L in the 1-mL waste stream or Process Blank sample.

The average percent recovery values over the three days ranged from 14 percent for the 25 ppm L spike in Blue to 143 percent for the 5 ppm L spike in Red. The 25 ppm spiked Process Blank sample was extracted and analyzed with the waste stream samples in Phase III to evaluate recovery efficiency. The recovery for these samples are given in Table 11 and summarized here.

Percent Recovery

	Process Blank	Blue	Red	Charcoal
HD	105	84	111	121
HN-1	86	62	94	86
L	138	14	104	105

The accuracy of the method was evaluated at the 25 ppm spike level by the difference between the percent recovery reported for the Process Blank and that of the various waste streams.

Method Accuracy

	Blue	Red	Charcoal
HD	21 percent low	6 percent high	16 percent high
HN-1	24 percent low	8 percent high	0 percent error
L	124 percent low*	34 percent low	33 percent low

* Since the recovery of the 25 ppm L-spiked Process Blank was greater than 100 percent, the difference between the Process Blank and the waste stream sample is greater than 100 percent.

Only the 25 ppm results are discussed here. Results for the other two spike levels are provided in Table 11, and these values can be compared to the spiked Process Blank samples in Table 7.

In addition to percent recovery information outlined above, the mean and standard deviation of the back-calculated concentrations in ppm over the three days are given in Table 11. The standard deviation values for the spiked waste stream extraction samples were used to establish the method detection limits and the method quantitation limits as stated on page 4 for the instrument limits. The sample size for the spiked waste stream samples is 6 over the three days. The standard deviation values for all three spiking levels are approximately the same, so the average standard deviation over the 3 spiking levels was used. This combination makes the sample size greater than 10.

Detection and Quantitation Limits for the Method

	Blue		Red		Charcoal	
	Method Det. Limit	**Method Quant. Limit**	**Method Det. Limit**	**Method Quant. Limit**	**Method Det. Limit**	**Method Quant. Limit**
HD	3 ppm	10 ppm	2 ppm	5 ppm	4 ppm	14 ppm
HN-1	4 ppm	12 ppm	2 ppm	7 ppm	2 ppm	6 ppm
L	NR*	NR*	14 ppm	46 ppm	25 ppm	85 ppm

* NR - Not Reported. Since essentially no L-Der was recovered in the Blue waste stream, the estimated values would have little meaning.

Two sets of limits have been estimated because the calibration standards are prepared differently than the waste stream samples. The term instrument detection limit (or detection limit for the instrument) refers to the 99 percent confidence limit for detecting the analyte when it is prepared from a pure stock in the extraction solvent mixture like the calibration standards. The method detection limit refers to the 99 percent confidence limit for detecting the analyte when it is extracted from a waste stream matrix using the procedure in the method being verified. The method detection limits are always higher than the instrument detection limits because additional variables are introduced.

Conclusions

The extraction method evaluated offers a reproducible procedure (RSD < 12 percent at 25 ppm) for the analysis of HD and HN-1 in RRS waste streams. When the results are evaluated using the spiked Process Blank samples, spike recovery errors range between 0 and 24 percent at 25 ppm for HD and HN-1. The method detection limit and method quantitation limit vary with agent and waste stream, but they are roughly 5 and 15 ppm, respectively, for both HD and HN-1. Five and 15 ppm may be used as conservative estimates of the method detection limit and method quantitation limit, respectively, for all HD and HN-1 waste stream analyses. It is not possible to establish composite limits for L since the limits for the Charcoal waste stream are larger than those for the Red waste stream.

Calibration standards, with simple linear regressions, may be used for HD and HN-1, but a quadratic regression model is required for the analysis of L-Der. However, the reported concentration for L in the Red waste stream is near the method quantitation limit and the concentration for L in the Charcoal waste stream is near the method detection limit.

The RRS waste stream matrix interferes with the analysis process. This interference increases the method detection limit and produces low recovery for L in spiked samples. The recovery of L is extremely low (124 percent lower than the 25 ppm Process Blank) in the Blue matrix (HN and L, however, are not present in the Blue neat HD ampules) and low in both the Red and Charcoal waste streams (34 and 33 percent at 25 ppm, respectively). A well trained operator is required for implementing this method, and judgments must be made about the character of the waste streams based upon the results. Accurate detection of L in a Blue waste stream is not possible, and underestimates of approximately 35 percent can be expected for both Red and Charcoal waste streams.

For agent recovery from Process Blank samples that are spiked at 25 ppm, there is an approximately 5 percent overestimated recovery for HD, 12 percent underestimated recovery for HN-1, and 30 percent overestimates of recovery for L (Tables 7 and 11). Except for HN-1, the recovery error increases at lower spike levels. Consequently, there are errors associated with the use of external calibration standards. The 25 ppm spike solutions were evaluated to assure that the recovery errors did not originate with the preparation of the spiked solutions. These solutions were diluted and analyzed without the extraction procedure. An independent set of calibration standards were prepared from the same original agent stock for the evaluation. The concentrations of the spike solutions were found to be within 4 percent of expected. During this analysis sequence, however, it was discovered that it was also necessary to correct for the concentration of the calibration standards prepared according to the method (see Attachment A, page B-18).

The stock solutions used throughout all phases of the Task 38 study, including those for dose confirmation and waste stream analyses, were prepared separately for each agent using chloroform as the solvent. There is no L-Der available for preparing calibration standards, so the L-Der was prepared from L stock as described in the method. The L-Der was washed with water to remove the HCl produced by the derivatization reaction and was then used as the stock for preparing calibration standards. With chloroform as solvent, it was not possible to wash the L-Der without loss. The first set of calibration standards was observed to have a large loss of L-Der in the final mixtures, so the first attempt to verify the method was not completed. After testing the L-Der preparation with just L, a second set of calibration standards was prepared using the procedure outlined in the method, and the verification process was repeated. In Phase II, during the second method verification process, it was again determined that the L-Der concentration was low. When a separate set of calibration standards was prepared (L-Der was separated from HD and HN-1, and the L-Der was not washed as specified in the method), the L-Der was determined to be 16.3 percent lower than expected. A 4.6 percent correction was also applied to the HD concentration during this process. Since there is no L-Der available for preparing calibration standards, these standards require careful evaluation.

One additional observation deserves brief attention. Low levels of L-Der, which could be detected and quantified in calibration standards at the beginning of an analysis sequence, could not be detected in the same sample when injected later. Likewise, in Phases II and III of this study, the calibration standards were analyzed at the beginning and at the end of the sequence, and in all cases the response ratio for the standards at the later time were lower than the values recorded at the beginning of the sequence. This degradation in analysis system performance must be monitored closely by the analyst. For the results presented in this report, the difference in system performance adds a degree of bias within the sequence since the average response ratio values were used in the regression routine.

The analysis performance for L is not adequate for routine analyses. Quantitative measurements of L may be restricted to certain matrices and may require a time-based GC/MS injection specification.

ATTACHMENT A

Analysis Method

1. Title

Interim GC-MS method for the determination of bis(2-chloroethyl)sulfide (HD), bis(2-chloroethyl)ethyl-amine (HN-1), and dichloro(2-chlorovinyl)arsine (L) in neutralization mixtures based on 1,3-dichloro-5,5-dimethylhydantoin (DCDMH) oxidation in organic solvent (RRS neutralization system).

Note on the Classification of this Method as "Interim"

Two circumstances require this method to be designated as "Interim" at the present time: 1. The recovery of L spiked into RRS neutralization solutions used to treat high levels of HD ("Blue" RRS scenario) is generally very low or zero, apparantly due to a minor RRS system decon product of HD that can react with derivatized L. The interim solution for this problem is to dilute the matrix with RRS solvent and re-analyze spiked and non-spiked aliquots. 2. There is presently insufficient experience with method calibration in general as well as spike recovery over a wide range of RRS neutralization samples to predict that method instructions and performance specifications in these areas will not need modification.

While the "Blue" RRS process involves treatment of HD only (i.e., analysis capability for L in spent "Blue" neutralization solutions is irrelevant), it cannot be ruled out that field use of the RRS system might involve a high HD/low L scenario since the chemical identity of all field treatment items may not be known. Therefore, adequate method performance for all three CW materials (HN-1, HD, and L) in all RRS trial scenarios ("Blue", "Red", "Charcoal", and "Vermiculite") is considered essential for qualification of this method, and work to characterize and solve this L analysis difficulty is on-going.

In addition, a formal Level 1 P&A study has not been undertaken for this Interim Method. Therefore, all information relating to method detection limits, method quantification limits, analysis ranges, and other calibration and method performance and QC measures, while based on data available to date, must be regarded as likely to be revised as more experience and data from the application of the method is obtained.

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3. Kev Words

Rapid Response System, RRS, 1,3-Dichloro-5,5-dimenhylhydantoin, DCMH, neurtalization solution, Vesicating Agent, Sulfur Mustard, HD, Nitrogen Mustard, HN-1, Lewisite, L, Gas chromatography-Mass Spectrometry, GC-MS

4. Revision History

Current Revision: 10-Jun.,'96 (DRAFT)
Previous Revisions: None
Original: 10-Jun.,'96 (DRAFT)

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6. Purpose and Application

The Rapid Response System (RRS) has been designed by non-stockpile chemical materials (NSCM) to neutralize chemical agents sourced from field training kits containing HD, HN-1 and L. These CW materials are typically in organic solution or are neat, contained in sealed glass ampules. In some cases, broken ampoules will have contaminated surrounding vermiculite packing material which would also be subjected to the RRS neutralization system. One additional RRS matrix is granulated charcoal which is either known or suspected to have been exposed to one or more of these three CWs. The analysis method described here is designed to apply to all such applications of the RRS neutralization system to all of these matrices. The method requires a GC-MS system capable of splitless evaporator cavity injection into a fused silica capillary column, analyte ionization by electron impact (EI), ion detection in the selected ion monitoring (SIM) mode, and a dedicated PC-based MS data processing capability. A Hewlett Packard Model 5971A MSD with Chemstation II data system was used for the development of the method, but any GC-MS system with approximately equivalent capabilities should be sufficient.

7. Analysis Range

HD and HN-1 are thought to have an analysis (quantitation) range from ~1 to 100 µg per mL (ppm) of spent RRS neutralization solution with a detection limit between 0.1and 0.5 ppm. The corresponding values for L are quantitation range of ~5 to 100 ppm and a detection limit between 0.5 and 2 ppm. A more accurate determination of these values will be made based on formal Level 1 P&A determinations planned for completion during Summer, 1996.

8. Summary of the Method

Method performance is documented for each sample through demonstration of the recovery from the spent neutralization solution of all three CW species spiked at an accurately known level, nominally 25 ppm. Because the anticipated spent neutralization solutions are expected to have residual oxidizing power, a direct spike into the sample, as received, is not meaningful. Therefore, both the residual oxidant and the strong acidity (HCl) anticipated in the spent neutralization solution is quenched prior to the spike. In addition, L must be derivatized prior to analysis by reaction with 1,3-propanedithiol (PDT) to form the arsenic disulfide derivative, 2-(2-chlorovinyl)-2-arsa-1,3-dithiacyclohexane [Cl-CH=CH-As(-SCH_2CH_2-CH_2S-)], i.e., L-PDT. An internal standard, *IS*, 1,2,4,5-tetrachlorabenzene, is used for CW quantitation as well as documentation of correct analyte GC retention. The sample is cleaned up by partitioning to aqueous buffer, and the extracted CW materials are analyzed without prior concentration of the extract. GC-MS analysis uses capillary gas chromatography, splitless sample injection, EI ionization, and SIM detection. Quantitation is against a calibration curve from GC-MS data for standards prepared in a simulated extract solvent. CW detection or non-detection is documented for each spent neutralization solution sample through the recovery of the corresponding CW material spiked into that sample. Inadequate recovery for these spiked samples requires dilution of the spent neutralization solution sample and re-analysis.

9. Materials and Equipment

a. *Reagents and Chemicals*

Reagent Water	KH_2PO_4	2,2,4-Trimethylpentane (Isooctane, i-C_8)
1,3-Propanedithiol (PDT)	K_2HPO_4	Thiolane (Tetrahydrothiophene)
1,2,4,5-Tetrachlorobenzene	tert-Butanol	Chloroform (HC stabilized)
Potassium Iodide (KI)	Acetic Acid	2,4-Dichloro-5,5-dimethylhydantoin (DCDMH)

In addition, SARM solutions of HN-1, HD and L are required to prepare calibration standards and solutions for spiking samples.

b. *Equipment*

GC-MS-Data System (HP 5971A MSD or other system with approximately equivalent or superior performance capability)

Disposable Glass Test Tubes, 16 mm x 125 mm (or functionally equivalent size), screw-cap with teflon cap liners

Syringes, 10 µL to 1,000 µL capacities

Solution Dispensers (three required), syringe pump type, up to 10-mL capacity (for dispensing 2.0 and 5.0 mL aliquots)

Pipettors and Tips (two required), 100 µL

Disposable Pipets, glass, 1.0 mL and 5.0 mL

Pipet Bulb

Disposable Transfer (Pasteur) Pipets, 5 ¾ in. size

Pipet Pump for Pasteur Pipets (or Latex Bulbs)

GC Autosampler Vials and Caps (with teflon-faced liners)

Bench-top Centrifuge (with rotor accommodating test tubes, above)

Vortex Mixer

Glass Reagent Bottles, 1-liter, screw cap with plastic or teflon liner (for the conc. phosphate buffer) and 1- to 2-oz with teflon liner for the *IS* spiking solution)

Volumetric glassware for preparing solutions (100 mL graduated cylinder, 1,000 and 25 mL volumetric flasks)

c. *Solutions*

Concentrated Phosphate Buffer, 1.0 M in K_2HPO_4 and 0.6 M in KH_2PO_4 (RT storage)

Dilute Phosphate Buffer, prepared fresh each day by diluting Phosphate Buffer 1:20, v:v, with reagent water (do not store)

1,2,4,5-tetrachlorobenzene, 1.00 mg/mL in isooctane (i-C_8), this is the *IS* spiking solution (RT storage)

PDT in i-C_8, 1.0 vol.%

XDS (RDT&E) stock solutions of HD, HN-1 and L, unspecified solvent, (for preparing calibration standards and matrix spikes)

RRS Solvent: 48.5:48.5:3 (v:v:v), chloroform:tert-butanol:water (RT storage)

RRS Neutralization Solution: ~ 0.55 M DCDMH, 10.8 grams DCDMH dissolved to 100 mL final volume in RRS solvent (~ -10°C or lower storage, 2-mo. storage life)

Water/Glacial Acetic Acid, 1:1, vol:vol

10. <u>Sample Work-Up Procedure</u>

(Note: "Sample" means either a spent neutralization solution aliquot or a process control (blank) aliquot; "vortex mix" means to briefly vortex mix and invert the tube at least twice—about a 5-sec procedure; samples are worked up in batches of 6 or fewer, depending on the centrifuge capacity with the work-up procedure described below performed essentially simultaneously on all samples of the batch; see No. 11 for the definition of a "set" of samples.)

a) *Approximate the relative oxidizing strength of the sample*: Make a comparison standard by adding a carefully dispensed single drop (Pasteur (dispo.) Pipet) of full strength RRS neutralization solution into a test tube containing a measured 3.0 mL of 1:1, water:glacial acetic acid and 100 to 300 mg of KI (visually approximated; added and dissolved just prior to the assay). The orange color that develops is I_3^- produced by the oxidizing capacity of the RRS neutralization system (DCDMH). Similarly assay the sample but continue to carefully add (counting) drops of sample until approximately the same orange color is achieved. However, do not add more than 20 drops. Record the approximate % original oxidizing power:

$$\% [Ox] = (1/ \# \text{ drops sample}) \times 100$$

If 20 drops still produce insufficient color density to match the reference color, approximate the fraction of reference color achieved to the nearest $1/5^{th}$ and adjust the %[Ox] value accordingly. For example, if the color density after 20 drops of the sample is 2/5 of the reference,

$$\% [Ox] = 2/5 \times 5\% = 2\%.$$

b) Pipet 1.0 mL of sample into a test tube of the specified type; use of a glass pipet (not a pipettor) is recommended; if a pipettor is used, sufficient rinse steps must be performed until the pipettor will not drip sample within 5 seconds after filling (typically, at least 5 rinses). Chill the sample in an ice/water bath at least 3 min.

c) Add 100µL thiolane (pipettor); vortex mix.

d) Add 20 µL *IS* solution (25-µL syringe); vortex mix.

e) Add 5 mL conc. phosphate buffer (dispenser pump); vortex mix.

f) Add 100 µL PDT solution (pipettor); vortex mix.

g) If the sample is to be spikes, add the spike aliquot at this point; add HD and HN-1 together or separately and L always separately and last; vortex mix after each spike addition.

h) Add 2 mL i-C_8; vigorously shake all tubes in the batch simultaneously in a horizontal position for 60 timed seconds.

i) Centrifuge just enough to cleanly separate the phases (typically, allowing the centrifuge to come to full speed and immediately shutting it off is sufficient); optionally, gravitational settling may be employed if both phases achieve clarity within ~2 min or less which is typical for blank and diluted samples. If the organic layer is on the bottom, add 0.5 mL i-C_8 and repeat the shake-out and centrifugation (settling).

j) Remove and discard the bottom (aqueous) layer using a dispo transfer pipet and the manipulator or bulb.

k) Add 5 mL dilute phosphate buffer (dispenser pump); shake (per *step h*) and centrifuge (per *step i*).

l) Fill and seal a GC autosampler vial with the upper (i-C_8) phase; discard the remaining sample to the lab decon management system.

11. Grouping of Samples into Sets

An analysis "set" consists of samples and process blanks (including spikes) plus accompanying calibration standards that are subjected to GC-MS analysis in a single autosampler setup and which will have their data worked up together. No more than 6 RRS spent neutralization solution samples should be grouped into an analysis set. For each spent neutralization solution sample, an identical aliquot spiked at an accurately known level (nominally 25 ppm) with each CW agent is included. Also included are process blanks consisting of RRS Solvent in spiked and non-spiked forms and a duplicate spiked and non-spiked set for one of the spent neutralization solution samples of the set (regardless of the number of samples in the set). Thus, if only one RRS spent neutralization solution sample is to be analyzed, the set will consist of 6 sample workups: one each spiked and non-spiked process blanks, two non-spiked aliquots, and two spiked aliquots (i.e., duplicates) of the spent neutralization solution sample. Similarly, a maximum size set (6 RRS spent neutralization solution samples) would result in 16 sample workups.

12. GC-MS Analysis

No data are available on the storage stability of sample extracts. Therefore, the interim position is that sample extracts should be analyzed on the same day (or that evening) that they are generated. If this is not possible, store them at ~-10°C or lower no more than 5 days prior to analysis. Replace the injector liner at least after every 50 injections of spent neutralization solution sample extracts (not blanks or calibration standards) or sooner depending on the rate of sample residue and septum debris build-up in the liner. Use only wide-bore liners designed for splitless injection. Liners used with rapid injection autosamplers (HP 7673, for example) must be packed with deactivated glass wool.

GC Conditions

Column	30 meter x 0.25 mm fused silica, 5% phenyl methylsilicone, 0.5 micron film (for example, Restek Rtx-5 #10238)
Carrier	Helium at 8 psi, pressure controlled
Purge (Splitter) Flow	30 to 50 mL/min
Injector Temp.	225°C
Transfer Line Temp.	275°C
Sample Injection	1.0 µL, splitless for 45 sec
Oven Program	65° (1 min hold); to 118° @ 15°/min (6 min hold); to 183° @ 12°/min (6 min hold); to 285° @ 25°/min (4 min hold). Total time is 30.03 min

MS Conditions

Ionization	70 eV, Electron Impact
Detection Mode	Selected Ion Monitoring (SIM), see Table 1 for monitored ions
Ion Source/EM gain	HP autotune at "high sensitivity" or manufacturer specification equivalent
SIM Parameters	Monitor 6 ions for each species; for HP MSD, dwell time is 50 msec; for other instruments, select functionally similar SIM conditions.
Filament On Delay	6 min

Table 1. SIM Ions and Confirmation Ion Abundances

Species	Example[a] GC Retention, min	Quant. Ion / *Conf. Ion (% of Quant. Ion)*[b] / Aux. Ions
HN-1	9.8	120 / *122 (35%), 154 (4%), 134 (5%)* / 92, 169
HD	10.6	158 / *109 (510%), 160 (68%), 111 (189%)* / 123, 96
IS	14.9	216 / *214 (78%), 218 (48%), 181 (19%)* / 220, 183
L-PDT	22.2	242 / *244 (35%), 181 (73%), 149 (117%)* / 165, 107

(a) Actual GC retention will vary somewhat in the individual application; see discussion below.
(b) Values for "% of Quant. Ion" are modifiable based on the analyst's experience with calibration standards.

The GC oven program is designed to give optimal separation from potential interferences and adequate time to SIM changeover between HN-1 and HD. The analyst may need to slightly vary the "hold" temperatures following the first and second temperature ramps in order to achieve elution of HD and L-PDT at the extreme end of their respective 6-min hold times. Alternatively, variation of the GC carrier supply pressure will have a similar effect. The requirement is that the peak top be located no earlier than one peak width nor later than two peak widths from the end of the respective 6-min hold period. The SIM ion changeover between HN-1 and HD should occur at a time nominally halfway between the two peak maxima.

13. Data Acceptance

GC-MS SIM Data—This method recognized that ion detection data acceptance always involves the analyst's judgment based on accumulating experience in the application of the method. However, this discretionary approach should operate within the following guidelines. In no cases are data to be accepted from spiked and non-spiked RRS spent neutralization solution samples without manual (i.e., direct visual) examination by the responsible analyst. This required data examination principally consists in verifying that peak areas appropriate to the level of CW material present are maximizing at the correct GC retention value and that they are present at approximately the correct ratios. The GC-MS data system can assist in this effort, but it cannot substitute for manual examination by the analyst. Often, this judgment must be made in conjunction with the analyst's recognition of overlapping or coeluting materials which interfere with the integrated peaks. In addition, the relative peak size criteria for confirmation and auxiliary ions (after accounting for apparent interferences) are expected to be less stringently met at lower levels of detection. For example, an auxiliary ion expected at 7% of the quantitation ion may be missing altogether for a bonafide detection which is supported by higher abundance confirmation or auxiliary ions. Final acceptance of data for the detection of HN-1, HD and L-PDT in the non-spiked aliquot for a given spent neutralization solution sample should always be justifiable by the chromatographic patterns observed for the corresponding spiked aliquot. Any analyst uncertainty in the qualification of data should be referred to the technical supervisor. Qualification of matrix sample data can be accomplished through hardcopy printouts of SIM chromatograms or by direct observation of them on the data system CRT. In the later case, data qualification which might be subject to dispute must de documented in hardcopy. This data acceptance process may result in reported detections which are below the P&A-generated method quantification limit; in these cases, the analyte is reported as "detected" but below the cited quantitation limit.

Spike Recoveries from Spent Neutralization Solution Samples—Spike recoveries from spent neutralization solution samples should be at least 80% for HN-1, 85% for HD, and at 70% for L and no greater than 110% for all three analytes. For HN-1 and HD, recoveries outside these ranges require a repeated sample workup and analysis (both spiked and non-spiked). In the event that the L spent neutralization solution sample spike recovery is less than 50% (as is expected for "Blue" scenario samples), dilute the sample 10x with RRS solvent and repeat the sample workup procedure and GC-MS analysis (both spiked and non-spiked). L analysis values for non-diluted samples with recoveries between 70% and 50% are adjusted for the actual L recovery found. For example, with the method quantification limit of L at 2 ppm, a sample with a non-detect on L and a 60% spiked L recovery would be reported as less than $2 \div 0.60 \cong 3$ ppm; if the same sample (L spike recovery of 60%) resulted in L quantified at 35 ppm the reported value should be $35 \div 0.60 = 58$ ppm. For 10x-diluted samples, a similar adjustment is applied (in addition to the 10x dilution factor) regardless of the L recovery obtained. In all cases, samples which require dilution for L analysis must be reported as such.

14. Calibration

Calibration is based on a 5-point calibration curve using standards with analyte concentrations corresponding to those that would be obtained in the ~2.6 mL sample extract from 100% recovery of analytes present in the starting 1.0-mL sample at the following levels: 1, 3, 10, 30, and 100 ppm. Thus, the actual concentrations of analytes in these calibration standards would not be 1, 3, 10, 30, and 100 ppm, but would be these values divided by 2.6. However, for convenience in preparing the calibration standards, a dilution factor of 2.5 is used. (Note, because quantification is based on an internal standard, this simplification is of absolutely no consequence for the correctness of the calibration obtained; also note that L is always detected as the derivative, L-PDT, but is quantified as the underivatized material). Thus the actual concentrations in the calibration solutions are 0.4, 1.2, 4, 12, and 40 µg/mL of CWAs and 8 µg/mL for the *IS* although the levels of identified to the data system are 1,3,10, 30, and 100 ppm of analyte and 20 ppm of *IS* in the starting neutralization solution sample. These standards are prepared in a solvent simulating the actual extract consisting of i-C_8:$CHCl_3$, 21:4, v:v. A stock solution of L-PDT is prepared from the L SARM in hydrocarbon solvent (hexane or i-C_8) by the addition of PDT at 5x the stoichiometric quantity and then washing the L-PDT once with unbuffered reagent water (to remove the HCl produced by the derivatization reaction). Once prepared, calibration solutions should be aliquoted to GC injection vials for storage at ~ -10°C or lower until use. The use of 200 µL vial inserts to make multiple sets of calibration standards is recommended. At this writing, no data are available on the storage stability of calibration standards under these conditions.

Because it is not expected that this method will be in constant (daily) use, a calibration verification approach to data quantification is not used. Instead, a complete set of calibration standards are run with each set of samples (as defined in No. 10, above) as the first five analyses, and then the second-lowest standard is repeated at the end of the analyses. Data from the first five standards are used to generate a calibration curve to quantify the set (using the *IS* approach), and the final standard at the end serves as a calibration check standard (i.e., it is quantified along with the other samples).

The issue of acceptance of calibration data cannot be addressed at this stage because of insufficient experience in application of the method. However, it is anticipated that the curves for HN-1 and L-PDT will not be linear, and a quadratic or higher order fit will be necessary.

15. Quantitation

Quantitation of HN-1, HD, and L-PDT detected in samples employs the internal standard method using the peak areas for the quantitation ions listed in Table 1 and calibration curves generated from the calibration standards described in the preceding section. The internal standard method uses the ratio of the peak area of each analyte's quantitation ion to the corresponding value for the internal standard. It is assumed that the analyst responsible for applying this method is thoroughly familiar with the standard application of the internal standard method for quantifying chromatographic data. Analysts who are not experienced in this regard are not qualified to perform the role as the responsible analyst.

16. Reporting Results

Because analysis results are not meaningful without their accompanying quality control data, reports of analysis results should include the following and be reported as complete analysis sets (see No. 11 for the definition of an analysis "set"):

a. The level found for each analyte in the non-spiked Process Blank and the spent neutralization solution samples included in the set. For the sample analyzed in duplicate, report the values obtained for each replicate as well as the average value.

b. The relative residual oxidative power value for each spent neutralization solution sample.

c. The percent recovery for the spiked Process Blank and the samples.

d. The calibration equation and correlation coefficient for each analyte.

e. The deviation (in %) of the end-of-set calibration check standard from its corresponding value with the other calibration standards.

f. The approximate signal to noise level for the quantification ion in the lowest calibration standard.

g. A narrative description of any exception to the method taken with the set as well as any observations which would have interpretative value for the validity of the results obtained (i.e., unusual patterns of interference with detection ions or other observations in the GC-MS data or the behavior of samples through the sample extraction/cleanup procedure not usually encountered with similar samples).

17. Quality Control

The most important quality control issues are addressed by method provisions for the analysis of a spiked aliquot for every sample with provisions for minimum spike recoveries (No. 13) and for the generation of a complete calibration curve with each analysis set. Some additional quality control requirements are the following:

Record Keeping—The following records will be maintained in order to document the quality of the data produced

a. A laboratory record book (LRB) will be used to record sample preparation activity including the preparation of standards and solutions and documentation of their storage conditions.

b. A GC-MS log book will be maintained. All instrument use (including documentation of the analysis of sample sets, traceable to the sample preparation LRB) and maintenance and repair activity will be recorded in this log book. Typically documentation of analysis sample sets consists of a loose-leaf binder with printouts of the autosampler setup and file names and directory name and location of the data in the GC-MS data system.

c. Hard copy print-outs of the mass spectrometer mass calibration report will be kept in a loose-leaf binder.

GC-MS System Maintenance—The GC-MS system manufacturer's recommendations on instrument maintenance are to be followed. In addition, criteria are given above for the routine changing of the GC injector liner. Decisions on the need for GC column replacement or removal of the inlet section are left to the judgment of the senior analyst responsible for the GC-MS data quality.

ATTACHMENT B

Results of Analytical Method Testing

TABLE 1. SIM RESULTS FOR CALIBRATION STANDARDS

Compound	Calib. Std. Group	Sample ID label	Actual concentration (ppm)	Internal standard target area	Target area	Response ratio
HD	1	070296A5.D	0.96	1,334,163	7,504	0.0056
		070296A4.D	2.88	1,307,442	24,705	0.0189
		070296A3.D	9.64	1,373,116	91,233	0.0664
		070296A2.D	28.8	1,354,492	292,494	0.2159
		070296A1.D	96.4	1,429,468	958,317	0.6704
HD	2	070296B5.D	0.96	1,323,466	7,970	0.0060
		070296B4.D	2.88	1,318,866	24,533	0.0186
		070296B3.D	9.64	1,338,262	85,472	0.0639
		070296B2.D	28.8	1,345,433	282,477	0.2100
		070296B1.D	96.4	1,367,267	929,955	0.6802
HD	3	070296C5.D	0.96	1,342,281	7,946	0.0059
		070296C4.D	2.88	1,313,458	23,353	0.0178
		070296C3.D	9.64	1,283,594	81,481	0.0635
		070296C2.D	28.8	1,360,067	266,266	0.1958
		070296C1.D	96.4	1,374,481	960,011	0.6985
HD	4	070296A4.D	2.88	1,307,442	24,705	0.0189
		070296B4.D	2.88	1,318,866	24,533	0.0186
		070296C4.D	2.88	1,313,458	23,353	0.0178
		0702964D.D	2.88	1,317,991	23,310	0.0177
		0702965D.D	2.88	1,354,225	25,219	0.0186
		0702966D.D	2.88	1,347,386	22,327	0.0166
		0702967D.D	2.88	1,371,883	23,992	0.0175
		0702968D.D	2.88	1,279,753	20,114	0.0157
		0702969D.D	2.88	1,266,112	20,088	0.0159
		0702960D.D	2.88	1,279,182	19,843	0.0155
HD	5	070296A4.D	2.88	1,307,442	24,705	0.0189
		07029602.D	2.88	1,254,267	21,291	0.0170
		07029603.D	2.88	1,243,035	19,884	0.0160
		07029604.D	2.88	1,251,490	20,590	0.0165
		07029605.D	2.88	1,208,038	20,894	0.0173
		07029606.D	2.88	1,231,799	20,561	0.0167
		07029607.D	2.88	1,253,065	20,045	0.0160
		07029608.D	2.88	1,214,023	20,351	0.0168
		07029609.D	2.88	1,221,995	20,528	0.0168
		07029610.D	2.88	1,247,921	18,913	0.0152

TABLE 1
(Continued)

Compound	Calib. Std. Group	Sample ID label	Actual concentration (ppm)	Internal standard target area	Target area	Response ratio
HN-1	1	070296A5.D	1.01	1,334,163	51,031	0.0382
		070296A4.D	3.03	1,307,442	130,290	0.0997
		070296A3.D	10.1	1,373,116	459,503	0.3346
		070296A2.D	30.3	1,354,492	1,442,222	1.0648
		070296A1.D	101	1,429,468	4,663,840	3.2626
HN-1	2	070296B5.D	1.01	1,323,466	52,160	0.0394
		070296B4.D	3.03	1,318,866	134,275	0.1018
		070296B3.D	10.1	1,338,262	438,380	0.3276
		070296B2.D	30.3	1,345,433	1,404,066	1.0436
		070296B1.D	101	1,367,267	4,533,495	3.3157
HN-1	3	070296C5.D	1.01	1,342,281	48,101	0.0358
		070296C4.D	3.03	1,313,458	129,495	0.0986
		070296C3.D	10.1	1,283,594	420,455	0.3276
		070296C2.D	30.3	1,360,067	1,325,658	0.9747
		070296C1.D	101	1,374,481	4,664,385	3.3936
HN-1	4	070296A4.D	3.03	1,307,442	130,290	0.0997
		070296B4.D	3.03	1,318,866	134,275	0.1018
		070296C4.D	3.03	1,313,458	129,495	0.0986
		0702964D.D	3.03	1,317,991	131,619	0.0999
		0702965D.D	3.03	1,354,225	135,133	0.0998
		0702966D.D	3.03	1,347,386	126,686	0.0940
		0702967D.D	3.03	1,371,883	129,448	0.0944
		0702968D.D	3.03	1,279,753	117,843	0.0921
		0702969D.D	3.03	1,266,112	114,380	0.0903
		0702960D.D	3.03	1,279,182	118,891	0.0929
HN-1	5	070296A4.D	3.03	1,307,442	130,290	0.0997
		07029602.D	3.03	1,254,267	115,506	0.0921
		07029603.D	3.03	1,243,035	115,206	0.0927
		07029604.D	3.03	1,251,490	118,330	0.0946
		07029605.D	3.03	1,208,038	114,183	0.0945
		07029606.D	3.03	1,231,799	115,391	0.0937
		07029607.D	3.03	1,253,065	114,141	0.0911
		07029608.D	3.03	1,214,023	110,930	0.0914
		07029609.D	3.03	1,221,995	116,171	0.0951
		07029610.D	3.03	1,247,921	111,061	0.0890

TABLE. 1
(Continued)

Compound	Calib. Std. Group	Sample ID label	Actual concentration (ppm)	Internal standard target area	Target area	Response ratio
L-Der	1	070296A5.D	0.81	1,334,163	1,876	0.0014
		070296A4.D	2.44	1,307,442	10,974	0.0084
		070296A3.D	8.12	1,373,116	77,039	0.0561
		070296A2.D	24.4	1,354,492	368,114	0.2718
		070296A1.D	81.2	1,429,468	1,604,612	1.1225
L-Der	2	070296B5.D	0.81	1,323,466	1,381	0.0010
		070296B4.D	2.44	1,318,866	11,026	0.0084
		070296B3.D	8.12	1,338,262	67,482	0.0504
		070296B2.D	24.4	1,345,433	346,551	0.2576
		070296B1.D	81.2	1,367,267	1,534,684	1.1224
L-Der	3	070296C5.D	0.81	1,342,281	1,398	0.0010
		070296C4.D	2.44	1,313,458	8,806	0.0067
		070296C3.D	8.12	1,283,594	55,232	0.0430
		070296C2.D	24.4	1,360,067	318,352	0.2341
		070296C1.D	81.2	1,374,481	1,560,130	1.1351
L-Der	4	070296A4.D	2.44	1,307,442	10,974	0.0084
		070296B4.D	2.44	1,318,866	11,026	0.0084
		070296C4.D	2.44	1,313,458	8,806	0.0067
		0702964D.D	2.44	1,317,991	9,354	0.0071
		0702965D.D	2.44	1,354,225	10,152	0.0075
		0702966D.D	2.44	1,347,386	9,689	0.0072
		0702967D.D	2.44	1,371,883	12,587	0.0092
		0702968D.D	2.44	1,279,753	7,117	0.0056
		0702969D.D	2.44	1,266,112	7,471	0.0059
		0702960D.D	2.44	1,279,182	6,387	0.0050
L-Der	5	070296A4.D	2.44	1,307,442	10,974	0.0084
		07029602.D	2.44	1,254,267	6,325	0.0050
		07029603.D	2.44	1,243,035	7,346	0.0059
		07029604.D	2.44	1,251,490	7,799	0.0062
		07029605.D	2.44	1,208,038	6,397	0.0053
		07029606.D	2.44	1,231,799	6,761	0.0055
		07029607.D	2.44	1,253,065	6,907	0.0055
		07029608.D	2.44	1,214,023	6,390	0.0053
		07029609.D	2.44	1,221,995	6,832	0.0056
		07029610.D	2.44	1,247,921	6,641	0.0053

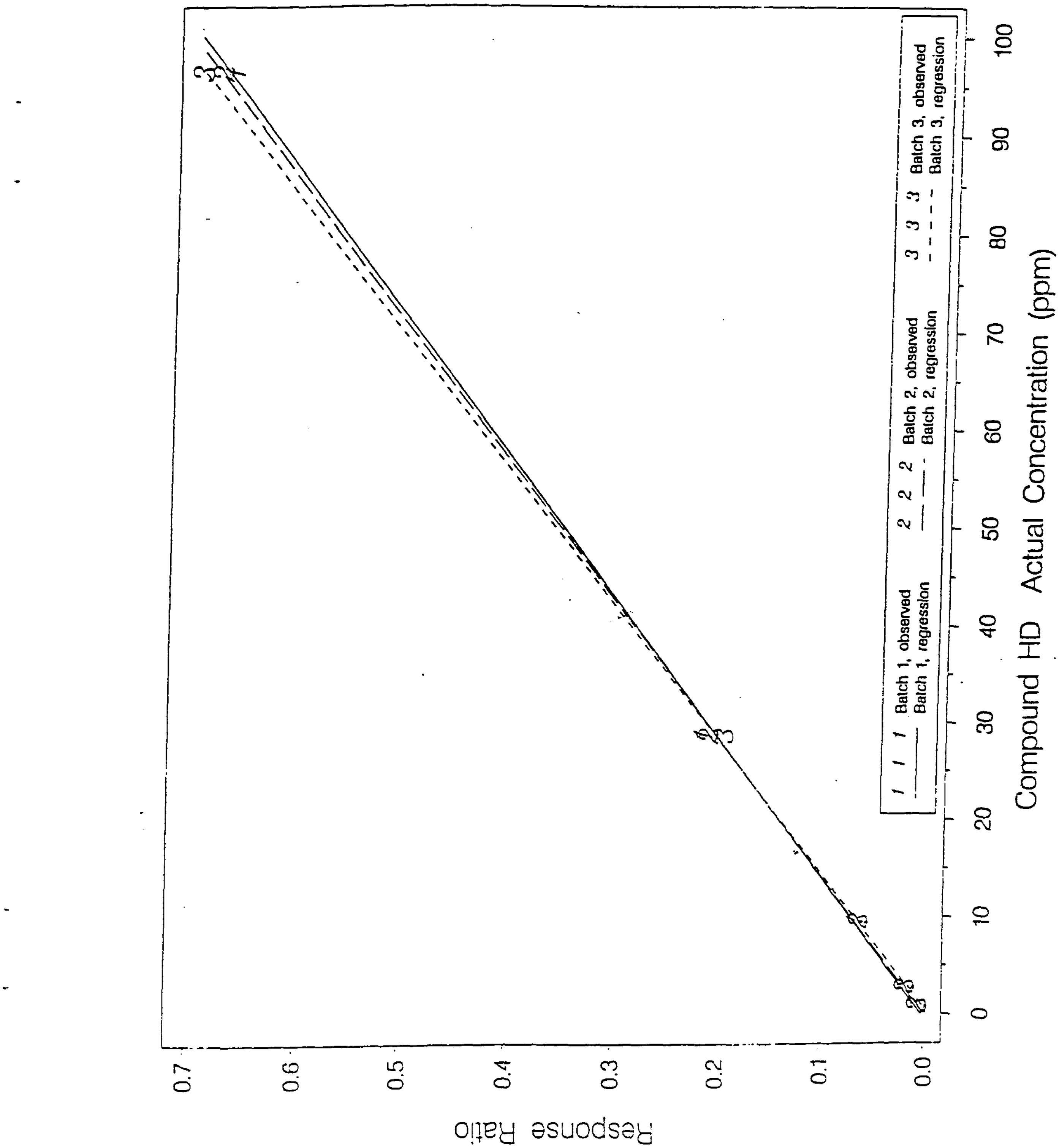


FIGURE 1. THREE BATCHES OF CALIBRATION STANDARDS FOR HD ON 7/2/96. OBSERVED RESPONSE RATIOS WITH FITTED LINEAR REGRESSIONS.

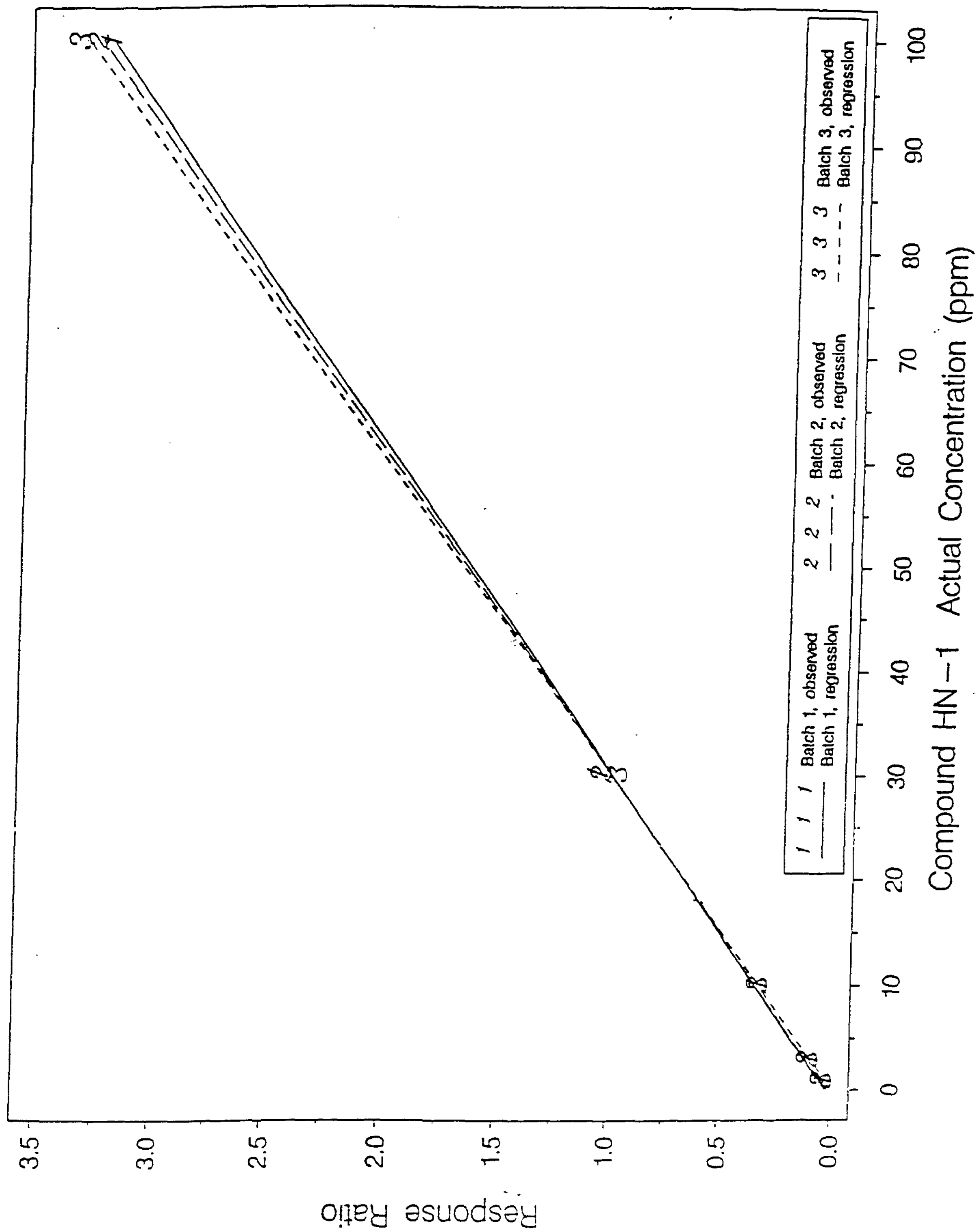


FIGURE 2. THREE BATCHES OF CALIBRATION STANDARDS FOR HN-1 ON 7/2/96. OBSERVED RESPONSE RATIOS WITH FITTED LINEAR REGRESSIONS.

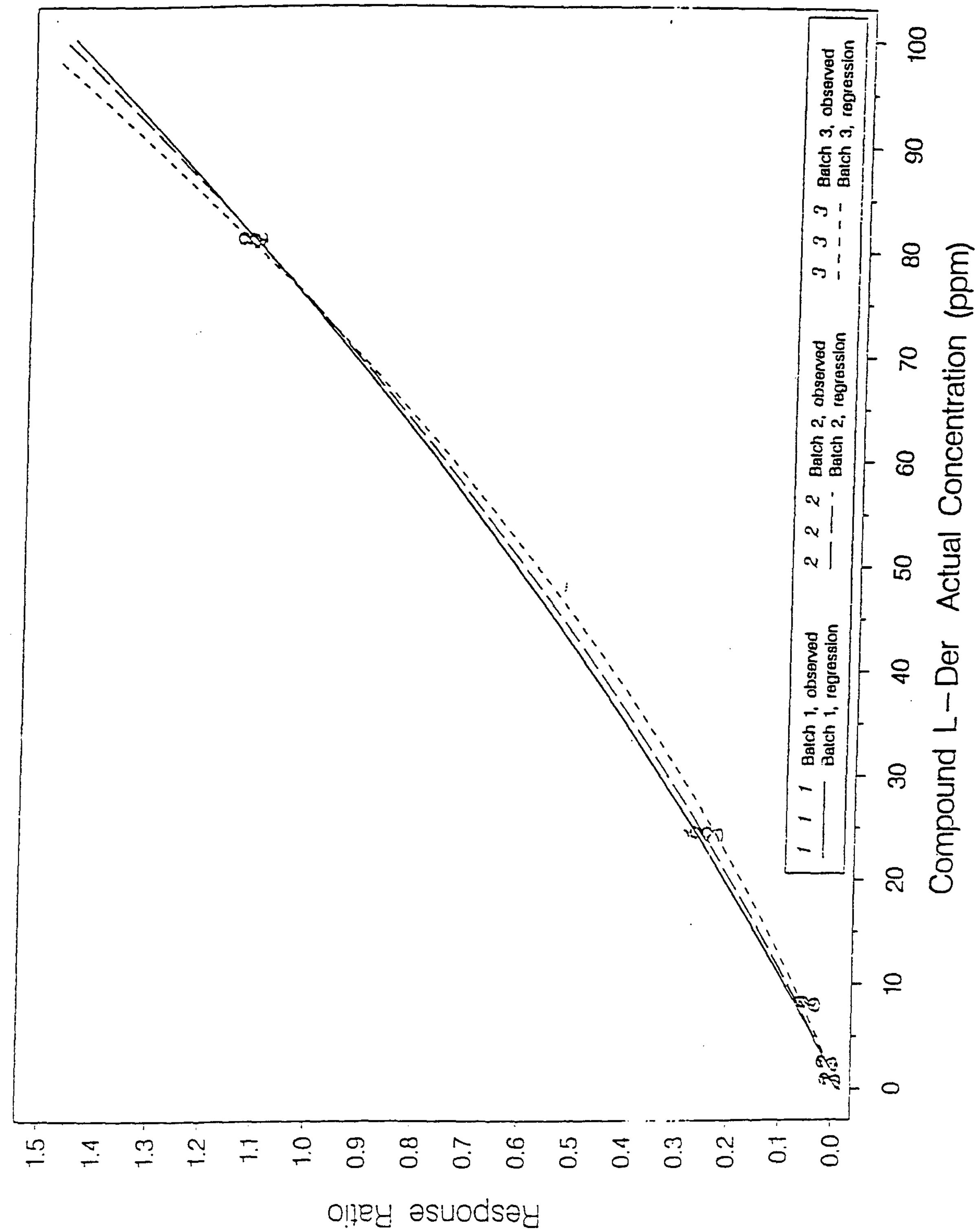


FIGURE 3. THREE BATCHES OF CALIBRATION STANDARDS FOR L-Der ON 7/2/96. OBSERVED RESPONSE RATIOS WITH FITTED QUADRATIC REGRESSIONS.

TABLE 2. RESULTS FOR THREE INDEPENDENT SETS OF CALIBRATION STANDARDS IN THE 1-100 ppm RANGE

Compound	Batch	Intercept	Std.Err., Intercept	Slope coefficient	Std.Err., Slope	Slope2 coefficient	Std.Err., Slope2	Deviation from. Regression
HD	1	0.002211	0.004778	0.00696751	0.000106	-	-	0.008439
	2	-.000604	0.002595	0.00707831	0.000057	-	-	0.004583
	3	-.005572	0.003291	0.00727766	0.000073	-	-	0.005813
	All	-.001322	0.002390	0.00710782	0.000053	-	-	0.007311
HN-1	1	0.021427	0.024070	0.03226638	0.000508	-	-	0.042492
	2	0.011112	0.014161	0.03281458	0.000299	-	-	0.024999
	3	-.012135	0.012193	0.03362845	0.000257	-	-	0.021525
	All	0.006801	0.011246	0.03290313	0.000237	-	-	0.034387
L-Der	1	-.017109	0.009859	0.01053678	0.001029	0.000043141	0.00001200	0.013552
	2	-.016020	0.009776	0.00964693	0.001021	0.000053922	0.00001190	0.013437
	3	-.013961	0.008822	0.00812863	0.000921	0.000074217	0.00001074	0.012126
	All	-.015697	0.005337	0.00943745	0.000557	0.000057093	0.00000650	0.012708

Regression lines or curves from the combined batches shown above as "All" are used to compute back-calculated calibration standard concentrations (ppm) shown in Tables 3 and 4.

TABLE 3. DESCRIPTIVE STATISTICS OF RESPONSE RATIOS FOR EACH COMPOUND AND CALIBRATION SAMPLE TYPE GROUPS OF TEN CALIBRATION STANDARDS AT 3 ppm

Sample Name=Ten separate sample preparations and injections at 3 ppm

Compound	Comparison standard (ppm)	Actual concentration (ppm)	N	Mean Response Ratio	Back-calculated concentration (ppm) Mean	S.D.	C.V	Instrument Detection Limit	Instrument Quantitation Limit
HD	3	2.88	10	0.0173	2.62	0.18	6.87	0.73	1.98
HN-1	3	3.03	10	0.0963	2.72	0.12	4.49	0.37	1.22
L-Der	3	2.44	10	0.0071	2.38	0.14	5.79	2.06	3.03

Sample Name=Ten injections from the same vial at 3 ppm

Compound	Comparison standard (ppm)	Actual concentration (ppm)	N	Mean Response Ratio	Back-calculated concentration (ppm) Mean	S.D.	C.V	Instrument Detection Limit	Instrument Quantitation Limit
HD	3	2.88	10	0.0167	2.54	0.14	5.46	0.60	1.57
HN-1	3	3.03	10	0.0934	2.63	0.09	3.35	0.26	0.88
L-Der	3	2.44	10	0.0058	2.25	0.10	4.45	1.95	2.65

TABLE 4. DESCRIPTIVE STATISTICS OF RESPONSE RATIOS FOR EACH AGENT AND CALIBRATION CONCENTRATION THREE INDEPENDENT CALIBRATION STANDARDS IN THE 1-100 ppm RANGE

Compound	Comparison standard (ppm)	Actual concentration (ppm)	N	Mean Response Ratio	Std. Dev.	Coef. of Variation	Back-calculated conc. (ppm)
HD	1	0.96	3	0.0059	0.0002	3.53	1.01
	3	2.88	3	0.0184	0.0006	3.14	2.78
	10	9.64	3	0.0646	0.0016	2.49	9.27
	30	28.80	3	0.2072	0.0104	5.00	29.34
	100	96.40	3	0.6830	0.0142	2.09	96.28
HN-1	1	1.01	3	0.0378	0.0018	4.82	0.94
	3	3.03	3	0.1000	0.0016	1.64	2.83
	10	10.10	3	0.3299	0.0041	1.24	9.82
	30	30.30	3	1.0277	0.0471	4.58	31.03
	100	101.00	3	3.3240	0.0658	1.98	100.82
L-Der	1	0.81	3	0.0012	0.0002	18.04	1.77
	3	2.44	3	0.0078	0.0010	12.35	2.46
	10	8.12	3	0.0499	0.0066	13.15	6.68
	30	24.40	3	0.2545	0.0190	7.48	24.88
	100	81.20	3	1.1267	0.0073	0.64	81.18

TABLE 5. RESULTS FOR THREE LEVELS OF SPIKED PROCESS BLANK SAMPLES

Compound	Sample Name	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
HN-1	Calibration Std	1.01	1,301,818	38,092	0.0293
		3.03	1,343,718	114,867	0.0855
		10.1	1,349,641	425,763	0.3155
		30.3	1,362,438	1,412,970	1.0371
		101	1,471,008	4,737,156	3.2203
HN-1	Process Blank	0.00	1,276,293	0	0.0000
HN-1	Process Blank	2.50	1,180,442	78,262	0.0663
		2.50	1,175,005	74,933	0.0638
		2.50	1,163,004	83,164	0.0715
		2.50	1,182,918	72,330	0.0611
		2.50	1,160,199	82,535	0.0711
		2.50	1,218,560	72,493	0.0595
		2.50	1,157,195	68,975	0.0596
HN-1	Process Blank	10.0	1,193,019	247,255	0.2073
		10.0	1,161,619	236,876	0.2039
		10.0	1,185,399	239,847	0.2023
		10.0	1,154,956	211,546	0.1832
		10.0	1,164,549	212,827	0.1828
		10.0	1,180,942	211,186	0.1788
		10.0	1,184,756	235,635	0.1989
HN-1	Process Blank	25.0	1,177,204	836,954	0.7110
		25.0	1,182,894	848,761	0.7175
		25.0	1,260,301	829,852	0.6585
		25.0	1,215,836	847,153	0.6968
		25.0	1,220,911	869,626	0.7123
		25.0	1,148,545	798,843	0.6955
		25.0	1,179,453	837,912	0.7104
HN-1	Calibration Std	1.01	1,350,594	25,517	0.0189
		3.03	1,308,467	84,988	0.0650
		10.1	1,372,819	391,447	0.2851
		30.3	1,365,829	1,310,296	0.9593
		101	1,519,061	4,650,198	3.0612

TABLE 5.
(Continued)

Compound	Sample Name	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
HD	Calibration Std	0.96	1,301,818	5,483	0.0042
		2.88	1,343,718	18,589	0.0138
		9.64	1,349,641	72,461	0.0537
		28.8	1,362,438	257,603	0.1891
		96.4	1,471,008	918,312	0.6243
HD	Process Blank	0.00	1,276,293	0	0.0000
HD	Process Blank	2.50	1,180,442	17,001	0.0144
		2.50	1,175,005	17,432	0.0148
		2.50	1,163,004	16,238	0.0140
		2.50	1,182,918	16,555	0.0140
		2.50	1,160,199	15,799	0.0136
		2.50	1,218,560	17,957	0.0147
		2.50	1,157,195	13,876	0.0120
HD	Process Blank	10.0	1,193,019	80,250	0.0673
		10.0	1,161,619	77,578	0.0668
		10.0	1,185,399	79,294	0.0669
		10.0	1,154,956	76,958	0.0666
		10.0	1,164,549	76,380	0.0656
		10.0	1,180,942	76,979	0.0652
		10.0	1,184,756	77,717	0.0656
HD	Process Blank	25.0	1,177,204	189,694	0.1611
		25.0	1,182,894	193,704	0.1638
		25.0	1,260,301	203,040	0.1611
		25.0	1,215,836	197,146	0.1621
		25.0	1,220,911	198,738	0.1628
		25.0	1,148,545	194,103	0.1690
		25.0	1,179,453	193,133	0.1637
HD	Calibration Std	0.96	1,350,594	3,724	0.0028
		2.88	1,308,467	12,169	0.0093
		9.64	1,372,819	64,606	0.0471
		28.8	1,365,829	239,519	0.1754
		96.4	1,519,061	941,300	0.6197

TABLE 5.
(Continued)

Compound	Sample Name	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
L-Der	Calibration Std	0.81	1,301,818	3,324	0.0026
		2.44	1,343,718	8,788	0.0065
		8.12	1,349,641	62,554	0.0463
		24.4	1,362,438	321,725	0.2361
		81.2	1,471,008	1,530,502	1.0404
L-Der	Process Blank	0.00	1,276,293	0	0.0000
L-Der	Process Blank	5.00	1,180,442	44,701	0.0379
		5.00	1,175,005	42,142	0.0359
		5.00	1,163,004	42,512	0.0366
		5.00	1,182,918	38,035	0.0322
		5.00	1,160,199	36,278	0.0313
		5.00	1,218,560	38,013	0.0312
		5.00	1,157,195	34,695	0.0300
L-Der	Process Blank	10.0	1,193,019	90,209	0.0756
		10.0	1,161,619	83,885	0.0722
		10.0	1,185,399	84,415	0.0712
		10.0	1,154,956	80,191	0.0694
		10.0	1,164,549	77,973	0.0670
		10.0	1,180,942	79,839	0.0676
		10.0	1,184,756	79,365	0.0670
L-Der	Process Blank	25.0	1,177,204	291,988	0.2480
		25.0	1,182,894	291,390	0.2463
		25.0	1,260,301	306,053	0.2428
		25.0	1,215,836	293,402	0.2413
		25.0	1,220,911	296,243	0.2426
		25.0	1,148,545	280,528	0.2442
		25.0	1,179,453	279,005	0.2366
L-Der	Calibration Std	0.81	1,350,594	0	0.0000
		2.44	1,308,467	2,171	0.0017
		8.12	1,372,819	25,139	0.0183
		24.4	1,365,829	181,746	0.1331
		81.2	1,519,061	1,311,522	0.8634

TABLE 6. REGRESSION RESULTS FOR CALIBRATION STANDARDS ANALYZED WITH SPIKED PROCESS BLANK SAMPLES

Compound	Batch	Intercept	Std.Err., Intercept	Slope coefficient	Std.Err. Slope	Slope2 coefficient	Std.Err., Slope2	Deviation from Regression
HD	1	-.004052	0.002509	0.00652828	0.000055	-	-	0.004432
	2	-.009979	0.002981	0.00651889	0.000066	-	-	0.005264
	Both	-.007016	0.002184	0.00652359	0.000048	-	-	0.005455
HN-1	1	0.007645	0.022323	0.03196795	0.000471	-	-	0.039408
	2	-.009863	0.016669	0.03052030	0.000352	-	-	0.029427
	Both	-.001109	0.021698	0.03124413	0.000458	-	-	0.054173
L-Der	1	-.014194	0.009218	0.00875368	0.000962	0.000052205	0.00001122	0.012671
	2	-.007591	0.005056	0.00344704	0.000528	0.000089676	0.00000615	0.006949
	Both	-.010893	0.028695	0.00610036	0.002996	0.000070941	0.00003493	0.055781

Regression lines or curves from the combined batches shown above as "Both" are used to compute back-calculated compound concentrations (ppm) shown in Table 7.

TABLE 7. DESCRIPTIVE STATISTICS FOR PROCESS BLANKS AND STANDARDS

Sample Name	Compound	Actual conc. of spike or std (ppm)	N	Mean Response Ratio	Std. Dev.	Coef. of Variation	Back-calculated conc. (ppm)	Percent Recovery
Calibration Standard	HD	0.96	2	0.0035	0.0010	29.52	1.61	-
		2.88	2	0.0116	0.0032	27.72	2.85	-
		9.64	2	0.0504	0.0047	9.30	8.80	-
		28.00	2	0.1822	0.0097	5.32	29.01	-
		96.40	2	0.6220	0.0033	0.52	96.42	-
Calibration Standard	HN-1	1.01	2	0.0241	0.0073	30.45	0.81	-
		3.03	2	0.0752	0.0145	19.30	2.44	-
		10.10	2	0.3003	0.0214	7.14	9.65	-
		30.30	2	0.9982	0.0550	5.51	31.98	-
		101.00	2	3.1408	0.1125	3.58	100.56	-
Calibration Standard	L-Der	0.81	2	0.0013	0.0018	141.42	1.95	-
		2.44	2	0.0041	0.0035	84.19	2.39	-
		8.12	2	0.0323	0.0198	61.32	6.58	-
		24.40	2	0.1846	0.0729	39.48	24.86	-
		81.20	2	0.9519	0.1252	13.15	81.18	-
Process Blank	HD	2.50	7	0.0139	0.0010	6.91	3.21	128.5
		10.00	7	0.0663	0.0008	1.21	11.24	112.4
		25.00	7	0.1634	0.0027	1.66	26.12	104.5
Process Blank	HN-1	2.50	7	0.0647	0.0051	7.90	2.11	84.3
		10.00	7	0.1939	0.0118	6.11	6.24	62.4
		25.00	7	0.7003	0.0202	2.88	22.45	89.8
Process Blank	L-Der	5.00	7	0.0336	0.0031	9.30	6.76	135.1
		10.00	7	0.0700	0.0032	4.60	11.68	116.8
		25.00	7	0.2431	0.0037	1.52	30.69	122.8

TABLE 8. RESULTS FOR THREE WASTE STREAM SAMPLES

Compound	Injection Date	Sample Name	Waste Stream	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
HN-1	07/08/95	Calibration Std		1.01	1,229,757	40,734	0.0331
				3.03	1,253,857	123,086	0.0982
				10.1	1,265,599	467,440	0.3693
				30.3	1,269,552	1,413,862	1.1137
				101	1,363,286	4,565,336	3.3488
HN-1	07/08/95	Extraction Sample	Blue	0.00	1,419,824	0	0.0000
				2.50	1,316,665	58,648	0.0445
				2.50	1,315,639	55,219	0.0420
				10.0	1,347,568	165,463	0.1228
				10.0	1,288,883	156,558	0.1215
				25.0	1,280,120	564,437	0.4409
				25.0	1,313,609	547,966	0.4171
HN-1	07/08/96	Extraction Sample	Red	0.00	1,206,279	0	0.0000
				2.50	1,099,030	88,003	0.0801
				2.50	1,077,885	88,950	0.0825
				10.0	1,142,997	244,528	0.2139
				10.0	1,099,497	234,215	0.2130
				25.0	1,107,504	796,953	0.7196
				25.0	1,054,513	795,011	0.7539
HN-1	07/08/96	Extraction Sample	Charcoal	0.00	1,175,556	0	0.0000
				2.50	1,107,506	87,397	0.0789
				2.50	1,075,616	100,531	0.0935
				10.0	1,081,556	222,253	0.2055
				10.0	1,107,330	211,902	0.1914
				25.0	1,100,883	738,416	0.6707
				25.0	1,069,669	756,576	0.7073
HN-1	07/08/96	Extraction Sample	Process Blank	0.00	1,107,402	0	0.0000
				25.0	1,053,244	705,033	0.6694
HN-1	07/08/96	Calibration Std		1.01	1,146,758	22,824	0.0199
				3.03	1,150,405	76,383	0.0664
				10.1	1,158,131	325,692	0.2812
				30.3	1,155,481	1,115,443	0.9653
				101	1,217,546	3,653,239	3.0005

TABLE 8.
(Continued)

Compound	Injection Date	Sample Name	Waste Stream	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
HN-1	07/10/96	Calibration Std		1.01	1,311,822	36,106	0.0275
				3.03	1,452,174	122,879	0.0846
				10.1	1,541,279	478,512	0.3105
				30.3	1,502,948	1,562,767	1.0398
				101	1,628,767	5,334,994	3.2755
HN-1	07/10/96	Extraction Sample	Blue	0.00	1,650,237	0	0.0000
				2.50	1,554,143	83,560	0.0538
				2.50	1,576,644	79,736	0.0506
				10.0	1,587,228	229,234	0.1444
				10.0	1,544,227	228,680	0.1481
				25.0	1,566,114	786,266	0.5020
				25.0	1,556,627	817,632	0.5253
HN-1	07/10/96	Extraction Sample	Red	0.00	1,532,021	0	0.0000
				2.50	1,393,392	122,449	0.0879
				2.50	1,434,419	124,377	0.0867
				10.0	1,374,428	335,281	0.2439
				10.0	1,312,407	327,737	0.2497
				25.0	1,323,184	1,061,874	0.8025
				25.0	1,327,626	1,039,955	0.7833
HN-1	07/10/96	Extraction Sample	Charcoal	0.00	1,311,100	0	0.0000
				2.50	1,231,359	106,876	0.0868
				2.50	1,247,644	102,155	0.0819
				10.0	1,240,262	290,664	0.2344
				10.0	1,215,439	281,071	0.2313
				25.0	1,179,525	836,801	0.7094
				25.0	1,371,298	955,995	0.6971
HN-1	07/10/96	Extraction Sample	Process Blank	0.00	1,259,030	0	0.0000
				25.0	1,172,221	858,465	0.7323
HN-1	07/10/96	Calibration Std		1.01	1,301,928	24,928	0.0191
				3.03	1,286,578	85,285	0.0663
				10.1	1,285,775	367,058	0.2855
				30.3	1,278,694	1,320,024	1.0323
				101	1,388,790	4,577,907	3.2963

TABLE 8.
(Continued)

Compound	Injection Date	Sample Name	Waste Stream	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
HN-1	07/11/96	Calibration Std		1.01	1,233,886	37,241	0.0302
				3.03	1,290,644	112,816	0.0874
				10.1	1,342,405	430,339	0.3206
				30.3	1,313,941	1,395,886	1.0624
				101	1,420,602	4,829,203	3.3994
HN-1	07/11/96	Extraction Sample	Blue	0.00	1,411,591	0	0.0000
				2.50	1,395,047	80,481	0.0577
				2.50	1,353,532	74,111	0.0548
				10.0	1,323,621	249,112	0.1882
				10.0	1,309,466	222,575	0.1700
				25.0	1,316,562	745,966	0.5666
				25.0	1,276,074	704,609	0.5522
HN-1	07/11/96	Extraction Sample	Red	0.00	1,245,975	0	0.0000
				2.50	1,112,913	97,146	0.0873
				2.50	1,142,971	99,191	0.0868
				10.0	1,147,977	270,555	0.2357
				10.0	1,131,470	271,787	0.2402
				25.0	1,143,115	856,216	0.7490
				25.0	1,056,520	788,226	0.7461
HN-1	07/11/96	Extraction Sample	Charcoal	0.00	1,082,582	0	0.0000
				2.50	1,052,507	98,663	0.0937
				2.50	1,001,573	87,095	0.0870
				10.0	1,009,983	213,022	0.2109
				10.0	939,402	203,360	0.2165
				25.0	975,486	702,250	0.7199
				25.0	974,223	650,953	0.6682
HN-1	07/11/96	Extraction Sample	Process Blank	0.00	962,527	0	0.0000
				25.0	886,001	610,223	0.6887
HN-1	07/11/96	Calibration Std		1.01	958,729	26,447	0.0276
				3.03	970,071	61,107	0.0630
				10.1	941,918	279,091	0.2963
				30.3	931,694	894,524	0.9601
				101	1,071,736	3,437,064	3.2070

TABLE 8.
(Continued)

Compound	Injection Date	Sample Name	Waste Stream	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
HD	07/08/96	Calibration Std		0.96	1,229,757	7,050	0.0057
				2.88	1,253,857	21,839	0.0174
				9.64	1,265,599	76,827	0.0607
				28.8	1,269,552	249,907	0.1968
				96.4	1,363,286	855,844	0.6278
HD	07/08/96	Extraction Sample	Blue	0.00	1,419,824	106,842	0.0753
				2.50	1,316,665	113,458	0.0862
				2.50	1,315,639	131,142	0.0997
				10.0	1,347,568	183,525	0.1362
				10.0	1,288,883	149,529	0.1160
				25.0	1,280,120	270,098	0.2110
				25.0	1,313,609	269,362	0.2051
HD	07/08/96	Extraction Sample	Red	0.00	1,206,279	0	0.0000
				2.50	1,099,030	16,280	0.0148
				2.50	1,077,885	15,740	0.0146
				10.0	1,142,997	80,354	0.0703
				10.0	1,099,497	77,406	0.0704
				25.0	1,107,504	189,123	0.1708
				25.0	1,054,513	189,235	0.1795
HD	07/08/96	Extraction Sample	Charcoal	0.00	1,175,556	0	0.0000
				2.50	1,107,506	7,215	0.0065
				2.50	1,075,616	6,560	0.0061
				10.0	1,081,556	67,948	0.0628
				10.0	1,107,330	97,109	0.0877
				25.0	1,100,883	206,404	0.1875
				25.0	1,069,669	201,605	0.1885
HD	07/08/96	Extraction Sample	Process Blank	0.00	1,107,402	0	0.0000
				25.0	1,053,244	172,527	0.1638
HD	07/08/96	Calibration Std		0.96	1,146,758	2,726	0.0024
				2.88	1,150,405	12,708	0.0110
				9.64	1,158,131	57,652	0.0498
				28.8	1,155,481	206,275	0.1785
				96.4	1,217,546	744,032	0.6111

TABLE 8.
(Continued)

Compound	Injection Date	Sample Name	Waste Stream	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
HD	07/10/96	Calibration Std		0.96	1,311,822	6,589	0.0050
				2.88	1,452,174	21,729	0.0150
				9.64	1,541,279	90,133	0.0585
				28.8	1,502,948	283,807	0.1888
				96.4	1,628,767	997,162	0.6122
HD	07/10/96	Extraction Sample	Blue	0.00	1,650,237	75,369	0.0457
				2.50	1,554,143	158,665	0.1021
				2.50	1,576,644	140,762	0.0893
				10.0	1,587,228	189,536	0.1194
				10.0	1,544,227	166,444	0.1078
				25.0	1,566,114	313,058	0.1999
				25.0	1,556,627	307,060	0.1973
HD	07/10/96	Extraction Sample	Red	0.00	1,532,021	0	0.0000
				2.50	1,393,392	22,963	0.0165
				2.50	1,434,419	21,986	0.0153
				10.0	1,374,428	104,361	0.0759
				10.0	1,312,407	103,064	0.0785
				25.0	1,323,184	242,313	0.1831
				25.0	1,327,626	237,547	0.1789
HD	07/10/96	Extraction Sample	Charcoal	0.00	1,311,100	0	0.0000
				2.50	1,231,359	14,313	0.0116
				2.50	1,247,644	5,616	0.0045
				10.0	1,240,262	117,391	0.0947
				10.0	1,215,439	86,793	0.0714
				25.0	1,179,525	241,822	0.2050
				25.0	1,371,298	281,017	0.2049
HD	07/10/96	Extraction Sample	Process Blank	0.00	1,259,030	0	0.0000
				25.0	1,172,221	203,156	0.1733
HD	07/10/96	Calibration Std		0.96	1,301,928	2,943	0.0023
				2.88	1,286,578	14,920	0.0116
				9.64	1,285,775	65,039	0.0506
				28.8	1,278,694	241,518	0.1889
				96.4	1,388,790	882,605	0.6355

TABLE 8.
(Continued)

Compound	Injection Date	Sample Name	Waste Stream	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
HD	07/11/96	Calibration Std		0.96	1,233,886	5,696	0.0046
				2.88	1,290,644	21,522	0.0167
				9.64	1,342,405	82,569	0.0615
				28.8	1,313,941	262,380	0.1997
				96.4	1,420,602	945,813	0.6658
HD	07/11/96	Extraction Sample	Blue	0.00	1,411,591	104,123	0.0738
				2.50	1,395,047	126,282	0.0905
				2.50	1,353,532	124,626	0.0921
				10.0	1,323,621	179,075	0.1353
				10.0	1,309,466	160,970	0.1229
				25.0	1,316,562	293,165	0.2227
				25.0	1,276,074	278,666	0.2184
HD	07/11/96	Extraction Sample	Red	0.00	1,245,975	0	0.0000
				2.50	1,112,913	18,817	0.0169
				2.50	1,142,971	18,651	0.0163
				10.0	1,147,977	88,840	0.0774
				10.0	1,131,470	89,223	0.0789
				25.0	1,143,115	209,593	0.1834
				25.0	1,056,520	189,297	0.1792
HD	07/11/96	Extraction Sample	Charcoal	0.00	1,082,582	0	0.0000
				2.50	1,052,507	12,895	0.0123
				2.50	1,001,573	12,299	0.0123
				10.0	1,009,983	66,175	0.0655
				10.0	939,402	62,123	0.0661
				25.0	975,486	186,163	0.1908
				25.0	974,223	186,282	0.1912
HD	07/11/96	Extraction Sample	Process Blank	0.00	962,527	0	0.0000
				25.0	886,001	151,386	0.1709
HD	07/11/96	Calibration Std		0.96	958,729	2,161	0.0023
				2.88	970,071	9,277	0.0096
				9.64	941,918	44,600	0.0474
				28.8	931,694	169,133	0.1815
				96.4	1,071,736	689,468	0.6433

TABLE 8.
(Continued)

Compound	Injection Date	Sample Name	Waste Stream	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
L-Der	07/08/96	Calibration Std		0.81	1,229,757	3,919	0.0032
				2.44	1,253,857	12,645	0.0101
				8.12	1,265,599	69,903	0.0552
				24.4	1,269,552	308,109	0.2427
				81.2	1,363,286	1,385,695	1.0164
L-Der	07/08/96	Extraction Sample	Blue	0.00	1,419,824	0	0.0000
				5.00	1,316,665	2,197	0.0017
				5.00	1,315,639	2,737	0.0021
				10.0	1,347,568	6,378	0.0047
				10.0	1,288,883	8,158	0.0063
				25.0	1,280,120	13,684	0.0107
				25.0	1,313,609	18,993	0.0145
L-Der	07/08/96	Extraction Sample	Red	0.00	1,206,279	226,406	0.1877
				5.00	1,099,030	253,290	0.2305
				5.00	1,077,885	276,356	0.2564
				10.0	1,142,997	360,404	0.3153
				10.0	1,099,497	434,218	0.3949
				25.0	1,107,504	442,814	0.3998
				25.0	1,054,513	524,429	0.4973
L-Der	07/08/96	Extraction Sample	Charcoal	0.00	1,175,556	295,461	0.2513
				5.00	1,107,506	320,802	0.2897
				5.00	1,075,616	335,573	0.3120
				10.0	1,081,556	393,428	0.3638
				10.0	1,107,330	393,603	0.3555
				25.0	1,100,883	524,548	0.4765
				25.0	1,069,669	583,422	0.5454
L-Der	07/08/96	Extraction Sample	Process Blank	0.00	1,107,402	0	0.0000
				25.0	1,053,244	320,081	0.3039
L-Der	07/08/96	Calibration Std		0.81	1,146,758	1,046	0.0009
				2.44	1,150,405	4,141	0.0036
				8.12	1,158,131	30,670	0.0265
				24.4	1,155,481	205,640	0.1780
				81.2	1,217,546	1,136,327	0.9333

TABLE 8.
(Continued)

Compound	Injection Date	Sample Name	Waste Stream	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
L-Der	07/10/96	Calibration Std		0.81	1,311,822	2,167	0.0017
				2.44	1,452,174	9,186	0.0063
				8.12	1,541,279	66,386	0.0431
				24.4	1,502,948	346,988	0.2309
				81.2	1,628,767	1,645,193	1.0101
L-Der	07/10/96	Extraction Sample	Blue	0.00	1,650,237	0	0.0000
				5.00	1,554,143	1,159	0.0007
				5.00	1,576,644	955	0.0006
				10.0	1,587,228	4,507	0.0028
				10.0	1,544,227	3,760	0.0024
				25.0	1,566,114	11,164	0.0071
				25.0	1,556,627	8,270	0.0053
L-Der	07/10/96	Extraction Sample	Red	0.00	1,532,021	328,115	0.2142
				5.00	1,393,392	364,171	0.2614
				5.00	1,434,419	440,722	0.3072
				10.0	1,374,428	466,507	0.3394
				10.0	1,312,407	449,077	0.3422
				25.0	1,323,184	772,965	0.5842
				25.0	1,327,626	682,025	0.5137
L-Der	07/10/96	Extraction Sample	Charcoal	0.00	1,311,100	100,708	0.0768
				5.00	1,231,359	199,744	0.1622
				5.00	1,247,644	80,075	0.0642
				10.0	1,240,262	165,545	0.1335
				10.0	1,215,439	221,708	0.1824
				25.0	1,179,525	373,331	0.3165
				25.0	1,371,298	621,268	0.4531
L-Der	07/10/96	Extraction Sample	Process Blank	0.00	1,259,030	0	0.0000
				25.0	1,172,221	384,564	0.3281
L-Der	07/10/96	Calibration Std		0.81	1,301,928	1,313	0.0010
				2.44	1,286,578	6,486	0.0050
				8.12	1,285,775	39,529	0.0307
				24.4	1,278,694	252,444	0.1974
				81.2	1,388,790	1,349,320	0.9716

TABLE 8.
(Continued)

Compound	Injection Date	Sample Name	Waste Stream	Actual conc. of spike or std (ppm)	Internal standard target area	Target area	Response ratio
L-Der	07/11/96	Calibration Std		0.81	1,233,886	2,519	0.0020
				2.44	1,290,644	10,491	0.0081
				8.12	1,342,405	65,315	0.0487
				24.4	1,313,941	301,411	0.2294
				81.2	1,420,602	1,436,699	1.0113
L-Der	07/11/96	Extraction Sample	Blue	0.00	1,411,591	0	0.0000
				5.00	1,395,047	3,775	0.0027
				5.00	1,353,532	4,981	0.0037
				10.0	1,323,621	11,229	0.0085
				10.0	1,309,466	11,816	0.0090
				25.0	1,316,562	32,910	0.0250
				25.0	1,276,074	25,252	0.0198
L-Der	07/11/96	Extraction Sample	Red	0.00	1,245,975	258,142	0.2072
				5.00	1,112,913	328,379	0.2951
				5.00	1,142,971	370,588	0.3242
				10.0	1,147,977	287,140	0.2501
				10.0	1,131,470	369,789	0.3268
				25.0	1,143,115	685,787	0.5999
				25.0	1,056,520	509,543	0.4823
L-Der	07/11/96	Extraction Sample	Charcoal	0.00	1,082,582	145,636	0.1345
				5.00	1,052,507	134,418	0.1277
				5.00	1,001,573	219,399	0.2191
				10.0	1,009,983	240,968	0.2386
				10.0	939,402	206,450	0.2198
				25.0	975,486	508,266	0.5210
				25.0	974,223	349,760	0.3590
L-Der	07/11/96	Extraction Sample	Process Blank	0.00	962,527	0	0.0000
				25.0	886,001	256,091	0.2890
L-Der	07/11/96	Calibration Std		0.81	958,729	0	0.0000
				2.44	970,071	3,558	0.0037
				8.12	941,918	19,218	0.0204
				24.4	931,694	129,540	0.1390
				81.2	1,071,736	948,877	0.8854

TABLE 9. RESULTS FOR CALIBRATING STANDARDS ANALYZED WITH THE WASTE STEAM SAMPLE

Compound	Injection Date	Intercept	Std.Err., Intercept	Slope coefficient	Std.Err., Slope	Slope2 coefficient	Std.Err., Slope2	Average Deviation from Regression
HD	07/08/96	-.003341	0.003076	0.00647066	0.000068	-	-	0.007683
	07/10/96	-.004171	0.002866	0.00652606	0.000063	-	-	0.007157
	07/11/96	-.006996	0.003524	0.00685843	0.000078	-	-	0.008801
HN-1	07/08/96	0.013514	0.042343	0.03149511	0.000893	-	-	0.105714
	07/10/96	-.008879	0.012842	0.03274973	0.000271	-	-	0.032063
	07/11/96	-.010830	0.023070	0.03287345	0.000487	-	-	0.057599
L-Der	07/08/96	-.011741	0.015611	0.00750617	0.001630	0.000057242	0.00001900	0.030347
	07/10/96	-.014030	0.008886	0.00769532	0.000928	0.000057693	0.00001082	0.017275
	07/11/96	-.009691	0.021921	0.00606664	0.002289	0.000070627	0.00002668	0.042613

Regression lines or curves on each respective date were used to compute back-calculated compound concentrations (ppm) shown in Table 10.

TABLE 10. DESCRIPTIVE STATISTICS FOR STANDARDS, PROCESS BLANK AND WASTE STREAMS

Compound	Sample Name	Injection Date	Actual conc. of spike or std (ppm)*	N	Mean Response Ratio	Std. Dev.	Coef. of Variation	Back-calculated conc. (ppm)	Percent Recovery
HD	Blue Waste Stream	07/08/96	0.0	1	0.0753	-	-	12.15	-
			2.5	2	0.0929	0.0096	10.28	14.88	109.3
			10.0	2	0.1261	0.0143	11.31	20.00	78.6
			25.0	2	0.2080	0.0042	2.02	32.67	82.1
HD	Blue Waste Stream	07/10/96	0.0	1	0.0457**	-	-	7.64	-
			2.5	2	0.0957	0.0091	9.47	15.30	306.5
			10.0	2	0.1136	0.0082	7.24	18.05	104.1
			25.0	2	0.1986	0.0019	0.94	31.07	93.7
HD	Blue Waste Stream	07/11/96	0.0	1	0.0738	-	-	11.78	-
			2.5	2	0.0913	0.0011	1.20	14.33	102.3
			10.0	2	0.1291	0.0087	6.77	19.85	80.7
			25.0	2	0.2205	0.0030	1.38	33.17	85.6
HD	Red Waste Stream	07/08/96	2.5	2	0.0147	0.0001	1.01	2.79	111.6
			10.0	2	0.0704	0.0001	0.10	11.39	113.9
			25.0	2	0.1751	0.0061	3.51	27.58	110.3
HD	Red Waste Stream	07/10/96	2.5	2	0.0159	0.0008	5.12	3.08	123.0
			10.0	2	0.0772	0.0018	2.38	12.47	124.7
			25.0	2	0.1810	0.0030	1.64	28.38	113.5
HD	Red Waste Stream	07/11/96	2.5	2	0.0166	0.0004	2.51	3.44	137.7
			10.0	2	0.0781	0.0010	1.33	12.41	124.1
			25.0	2	0.1813	0.0030	1.63	27.45	109.8
HD	Charcoal Waste Stream	07/08/96	2.5	2	0.0063	0.0003	4.66	1.49	59.6
			10.0	2	0.0753	0.0176	23.37	12.15	121.5
			25.0	2	0.1880	0.0007	0.37	29.57	118.3
HD	Charcoal Waste Stream	07/10/96	2.5	2	0.0081	0.0050	62.47	1.87	75.0
			10.0	2	0.0830	0.0164	19.79	13.36	133.6
			25.0	2	0.2050	0.0001	0.03	32.05	128.2
HD	Charcoal Waste Stream	07/11/96	2.5	2	0.0123	0.0000	0.16	2.81	112.3
			10.0	2	0.0658	0.0004	0.65	10.62	106.2
			25.0	2	0.1910	0.0003	0.14	28.87	115.5

TABLE 10.
(Continued)

Compound	Sample Name	Injection Date	Actual conc. of spike or std (ppm)	N	Mean Response Ratio	Std. Dev.	Coef. of Variation	Back-calculated conc. (ppm)	Percent Recovery
HD	Calibration Standard	07/08/96	0.96	2	0.0041	0.0024	58.52	1.14	-
			2.88	2	0.0142	0.0045	31.65	2.72	-
			9.64	2	0.0552	0.0077	13.98	9.05	-
			28.80	2	0.1877	0.0130	6.91	29.52	-
			96.40	2	0.6194	0.0118	1.91	96.25	-
HD	Calibration Standard	07/10/96	0.96	2	0.0036	0.0020	53.64	1.20	-
			2.88	2	0.0133	0.0024	17.93	2.67	-
			9.64	2	0.0545	0.0056	10.24	9.00	-
			28.80	2	0.1889	0.0000	0.02	29.58	-
			96.40	2	0.6239	0.0165	2.64	96.24	-
HD	Calibration Standard	07/11/96	0.96	2	0.0034	0.0017	48.63	1.52	-
			2.88	2	0.0131	0.0050	38.33	2.93	-
			9.64	2	0.0544	0.0100	18.39	8.96	-
			28.80	2	0.1906	0.0128	6.74	28.81	-
			96.40	2	0.6546	0.0159	2.43	96.46	-
HD	Process Blark	07/08/96	25.00	1	0.1638	-	-	25.83	103.3
		07/10/96	25.00	1	0.1733	-	-	27.20	108.8
		07/11/96	25.00	1	0.1709	-	-	25.93	103.7

TABLE 10.
(Continued)

Compound	Sample Name	Injection Date	Actual conc. of spike or std (ppm)	N	Mean Response Ratio	Std. Dev.	Coef. of Variation	Back-calculated conc. (ppm)	Percent Recovery
HN-1	Calibration Standard	07/08/96	1.01	2	0.0265	0.0093	35.26	0.41	-
			3.03	2	0.0823	0.0225	27.30	2.18	-
			10.10	2	0.3253	0.0623	19.16	9.90	-
			30.30	2	1.0395	0.1049	10.09	32.58	-
			101.00	2	3.1746	0.2463	7.76	100.37	-
HN-1	Calibration Standard	07/10/96	1.01	2	0.0233	0.0059	25.38	0.98	-
			3.03	2	0.0755	0.0130	17.18	2.58	-
			10.10	2	0.2980	0.0177	5.93	9.37	-
			30.30	2	1.0361	0.0053	0.51	31.91	-
			101.00	2	3.2859	0.0147	0.45	100.60	-
HN-1	Calibration Standard	07/11/96	1.01	2	0.0289	0.0018	6.36	1.21	-
			3.03	2	0.0752	0.0173	22.96	2.62	-
			10.10	2	0.3084	0.0172	5.56	9.71	-
			30.30	2	1.0112	0.0723	7.15	31.09	-
			101.00	2	3.3032	0.1360	4.12	100.81	-
HN-1	Process Blank	07/08/96	25.00	1	0.6694	-	-	20.82	83.3
		07/10/96	25.00	1	0.7323	-	-	22.63	90.5
		07/11/96	25.00	1	0.6887	-	-	21.28	85.1

TABLE 10.
(Continued)

Compound	Sample Name	Injection Date	Actual conc. of spike or std (ppm)	N	Mean Response Ratio	Std. Dev.	Coef. of Variation	Back-calculated conc. (ppm)	Percent Recovery
HN-1	Blue Waste Stream	07/08/96	2.5	2	0.0433	0.0018	4.20	0.94	37.8
			10.0	2	0.1221	0.0009	0.76	3.45	34.5
			25.0	2	0.4290	0.0168	3.92	13.19	52.8
HN-1	Blue Waste Stream	07/10/96	2.5	2	0.0522	0.0023	4.33	1.86	74.6
			10.0	2	0.1463	0.0026	1.77	4.74	47.4
			25.0	2	0.5137	0.0164	3.20	15.96	63.8
HN-1	Blue Waste Stream	07/11/96	2.5	2	0.0562	0.0021	3.69	2.04	81.6
			10.0	2	0.1791	0.0129	7.20	5.78	57.8
			25.0	2	0.5594	0.0102	1.82	17.35	69.4
HN-1	Red Waste Stream	07/08/96	2.5	2	0.0813	0.0017	2.13	2.15	86.1
			10.0	2	0.2135	0.0006	0.30	6.35	63.5
			25.0	2	0.7368	0.0243	3.29	22.96	91.9
HN-1	Red Waste Stream	07/10/96	2.5	2	0.0873	0.0008	0.95	2.94	117.5
			10.0	2	0.2468	0.0041	1.66	7.81	78.1
			25.0	2	0.7929	0.0136	1.71	24.48	97.9
HN-1	Red Waste Stream	07/11/96	2.5	2	0.0870	0.0004	0.41	2.98	119.1
			10.0	2	0.2379	0.0032	1.35	7.57	75.7
			25.0	2	0.7475	0.0021	0.28	23.07	92.3
HN-1	Charcoal Waste Stream	07/08/96	2.5	2	0.0862	0.0103	11.94	2.31	92.3
			10.0	2	0.1984	0.0100	5.04	5.87	58.7
			25.0	2	0.6890	0.0258	3.75	21.45	85.8
HN-1	Charcoal Waste Stream	07/10/96	2.5	2	0.0843	0.0035	4.12	2.85	113.9
			10.0	2	0.2328	0.0022	0.94	7.38	73.8
			25.0	2	0.7033	0.0087	1.24	21.75	87.0
HN-1	Charcoal Waste Stream	07/11/96	2.5	2	0.0903	0.0048	5.31	3.08	123.1
			10.0	2	0.2137	0.0039	1.84	6.83	68.3
			25.0	2	0.6940	0.0366	5.27	21.44	85.8

TABLE 10.
(Continued)

Compound	Sample Name	Injection Date	Actual conc. of spike or std (ppm)	N	Mean Response Ratio	Std. Dev.	Coef. of Variation	Back-calculated conc. (ppm)	Percent Recovery
L-Der	Blue Waste Stream	07/08/96	5	2	0.0019	0.0003	15.53	1.79	35.8
			10	2	0.0055	0.0011	20.41	2.26	22.6
			25	2	0.0126	0.0027	21.20	3.16	12.7
L-Der	Blue Wastn Stream	07/10/96	5	2	0.0007	0.0001	14.65	1.88	37.7
			10	2	0.0026	0.0003	10.85	2.13	21.3
			25	2	0.0062	0.0013	20.64	2.58	10.3
L-Der	Blue Waste Stream	07/11/96	5	2	0.0032	0.0007	21.57	2.07	41.5
			10	2	0.0088	0.0004	4.36	2.94	29.4
			25	2	0.0224	0.0037	16.45	5.00	20.0
L-Der	Red Waste Stream	07/08/96	0	1	0.1877	-	-	22.65	-
			5	2	0.2434	0.0183	7.53	28.01	107.1
			10	2	0.3551	0.0563	15.85	37.91	152.6
			25	2	0.4486	0.0689	15.37	45.52	91.5
L-Der	Red Waste Stream	07/10/96	0	1	0.2142	-	-	24.98	-
			5	2	0.2843	0.0325	11.41	31.38	128.1
			10	2	0.3408	0.0020	0.57	36.26	112.8
			25	2	0.5489	0.0498	9.08	52.50	110.1
L-Der	Red Waste Stream	07/11/96	0	1	0.2072	-	-	27.16	-
			5	2	0.3096	0.0206	6.66	36.84	193.6
			10	2	0.2885	0.0542	18.80	34.94	77.8
			25	2	0.5411	0.0832	15.37	55.25	112.4
L-Der	Charcoal Waste Stream	07/08/96	0	1	0.2513	-	-	28.75	-
			5	2	0.3008	0.0158	5.25	33.22	89.5
			10	2	0.3596	0.0059	1.63	38.29	95.4
			25	2	0.5110	0.0488	9.54	50.32	86.3
L-Der	Charcoal Waste Stream	07/10/96	0	1	0.0768	-	-	10.91	-
			5	2	0.1132	0.0693	61.24	14.87	79.2
			10	2	0.1579	0.0346	21.91	19.50	85.9
			25	2	0.3848	0.0965	25.09	39.89	115.9
L-Der	Charcoal Waste Stream	07/11/96	0	1	0.1345	-	-	19.39	-
			5	2	0.1734	0.0646	37.25	23.66	85.3
			10	2	0.2292	0.0133	5.81	29.35	99.5
			25	2	0.4400	0.1146	26.04	47.67	113.1

TABLE 10.
(Continued)

Compound	Sample Name	Injection Date	Actual conc. of spike or std (ppm)	N	Mean Response Ratio	Std. Dev.	Coef. of Variation	Back-calculated conc. (ppm)	Percent Recovery
L-Der	Calibration Standard	07/08/96	0.81	2	0.0020	0.0016	78.48	1.81	-
			2.44	2	0.0068	0.0046	67.02	2.43	-
			8.12	2	0.0409	0.0203	49.76	6.67	-
			24.40	2	0.2103	0.0458	21.76	24.87	-
			81.20	2	0.9749	0.0588	6.03	81.18	-
L-Der	Calibration Standard	07/10/96	0.81	2	0.0013	0.0005	34.20	1.97	-
			2.44	2	0.0057	0.0009	15.98	2.51	-
			8.12	2	0.0369	0.0087	23.62	6.32	-
			24.40	2	0.2141	0.0237	11.04	24.98	-
			81.20	2	0.9908	0.0272	2.75	81.18	-
L-Der	Calibration Standard	07/11/96	0.81	2	0.0010	0.0014	141.42	1.73	-
			2.44	2	0.0059	0.0032	53.48	2.50	-
			8.12	2	0.0345	0.0200	57.86	6.76	-
			24.40	2	0.1842	0.0639	34.68	24.80	-
			81.20	2	0.9483	0.0891	9.39	81.19	-
L-Der	Process Blank	07/08/96	25.00	1	0.3039	-	-	33.50	134.0
		07/10/96	25.00	1	0.3281	-	-	35.18	140.7
		07/11/96	25.00	1	0.2890	-	-	34.99	140.0

* When no agent was detected at the 0 ppm level(no spike)in the waste stream, the 0 ppm level is not included in this table.

** Value rejected based on the Q test. GC injection problem for this sample. Therefore, the HD results for this day are not included in the average values reported in Table 11.

TABLE 11. SUMMARY OF DESCRIPTIVE STATISTICS FOR WASTE STREAM SAMPLES OVER THREE DAYS OF ANALYSIS

Compound	Sample Name	Actual conc. of spike or std (ppm)*	N	Mean Response Ratio	Back-calculated Conc. (ppm) Mean	Std.Dev.	C.V.	Percent Recovery	Std.Dev., Percent Recovery
HD	Calibration Standard	0.96	6	0.0037	1.29	0.30	23.3	-	-
		2.88	6	0.0135	2.77	0.50	17.9	-	-
		9.64	6	0.0547	9.00	0.93	10.3	-	-
		28.80	6	0.1890	29.30	1.28	4.4	-	-
		96.40	6	0.6326	96.31	1.74	1.8	-	-
HD	Process Blank	25.00	3	0.1693	26.32	0.76	2.9	105.3	3.0
HD	Blue Waste Stream	0.00	2	0.0745	11.96	0.26	2.2	-	-
		2.50	4	0.0921	14.60	0.91	6.3	105.8	34.5
		10.00	4	0.1276	19.92	1.47	7.4	79.6	14.8
		25.00	4	0.2143	32.92	0.54	1.6	83.8	2.7
HD	Red Waste Stream	2.50	6	0.0157	3.10	0.30	9.7	124.1	12.0
		10.00	6	0.0752	12.09	0.56	4.7	120.9	5.6
		25.00	6	0.1791	27.80	0.68	2.4	111.2	2.7
HD	Charcoal Waste Stream	2.50	6	0.0089	2.06	0.70	33.9	82.3	27.9
		10.00	6	0.0747	12.04	2.06	17.1	120.4	20.6
		25.00	6	0.1947	30.16	1.49	5.0	120.7	6.0

TABLE 11.
(Continued)

Compound	Sample Name	Actual conc. of spike or std (ppm)	N	Mean Response Ratio	Back-calculated Conc. (ppm) Mean	Std.Dev.	C.V.	Percent Recovery	Std.Dev., Percent Recovery
HN-1	Calibration Standard	1.01	6	0.0262	0.87	0.40	46.0	-	-
		3.03	6	0.0776	2.46	0.48	19.7	-	-
		10.10	6	0.3106	9.66	0.98	10.1	-	-
		30.30	6	1.0289	31.86	1.91	6.0	-	-
		101.00	6	3.2546	100.60	3.97	3.9	-	-
HN-1	Process Blank	25.00	3	0.6968	21.58	0.94	4.4	86.3	3.8
HN-1	Blue Waste Stream	2.50	6	0.0505	1.62	0.53	32.7	64.6	21.1
		10.00	6	0.1492	4.65	1.06	22.7	46.5	10.6
		25.00	6	0.5007	15.50	1.92	12.4	62.0	7.7
HN-1	Red Waste Stream	2.50	6	0.0852	2.69	0.42	15.5	107.5	16.7
		10.00	6	0.2328	7.24	0.70	9.7	72.4	7.0
		25.00	6	0.7591	23.51	0.85	3.6	94.0	3.4
HN-1	Charcoal Waste Stream	2.50	6	0.0870	2.74	0.39	14.2	109.8	15.6
		10.00	6	0.2150	6.69	0.70	10.5	66.9	7.0
		25.00	6	0.6955	21.55	0.65	3.0	86.2	2.6

TABLE 11.
(Continued)

Compound	Sample Name	Actual conc. of spike or std (ppm)	N	Mean Response Ratio	Back-calculated Conc. (ppm) Mean	Std.Dev.	C.V.	Percent Recovery	Std.Dev., Percent Recovery
L-Der	Calibration Standard	0.81	6	0.0015	1.84	0.18	9.6	-	-
		2.44	6	0.0061	2.48	0.35	14.1	-	-
		8.12	6	0.0374	6.56	1.76	26.8	-	-
		24.40	6	0.2029	24.80	3.73	15.0	-	-
		81.20	6	0.9713	81.16	2.85	3.5	-	-
L-Der	Process Blank	25.00	3	0.3070	34.55	0.92	2.7	138.2	3.7
L-Der	Blue Waste Stream	5.00	6	0.0019	1.92	0.14	7.3	38.3	2.8
		10.00	6	0.0056	2.44	0.39	16.1	24.4	3.9
		25.00	6	0.0137	3.58	1.17	32.5	14.3	4.7
L-Der	Red Waste Stream	0.00	3	0.2030	24.93	2.25	9.0	-	-
		5.00	6	0.2791	32.06	4.33	13.5	142.7	52.8
		10.00	6	0.3281	36.32	3.35	9.2	113.9	45.5
		25.00	6	0.5129	51.03	5.98	11.7	104.4	18.9
L-Der	Charcoal Waste Stream	0.00	3	0.1542	19.68	8.92	45.3	-	-
		5.00	6	0.1958	23.80	9.45	39.7	89.7	81.3
		10.00	6	0.2489	29.03	8.59	29.6	93.5	18.0
		25.00	6	0.4453	45.83	7.41	16.2	104.6	26.5

* When no agent was detected on the 0 ppm level (no spike) in the waste stream, the 0 ppm level is not included in this table.

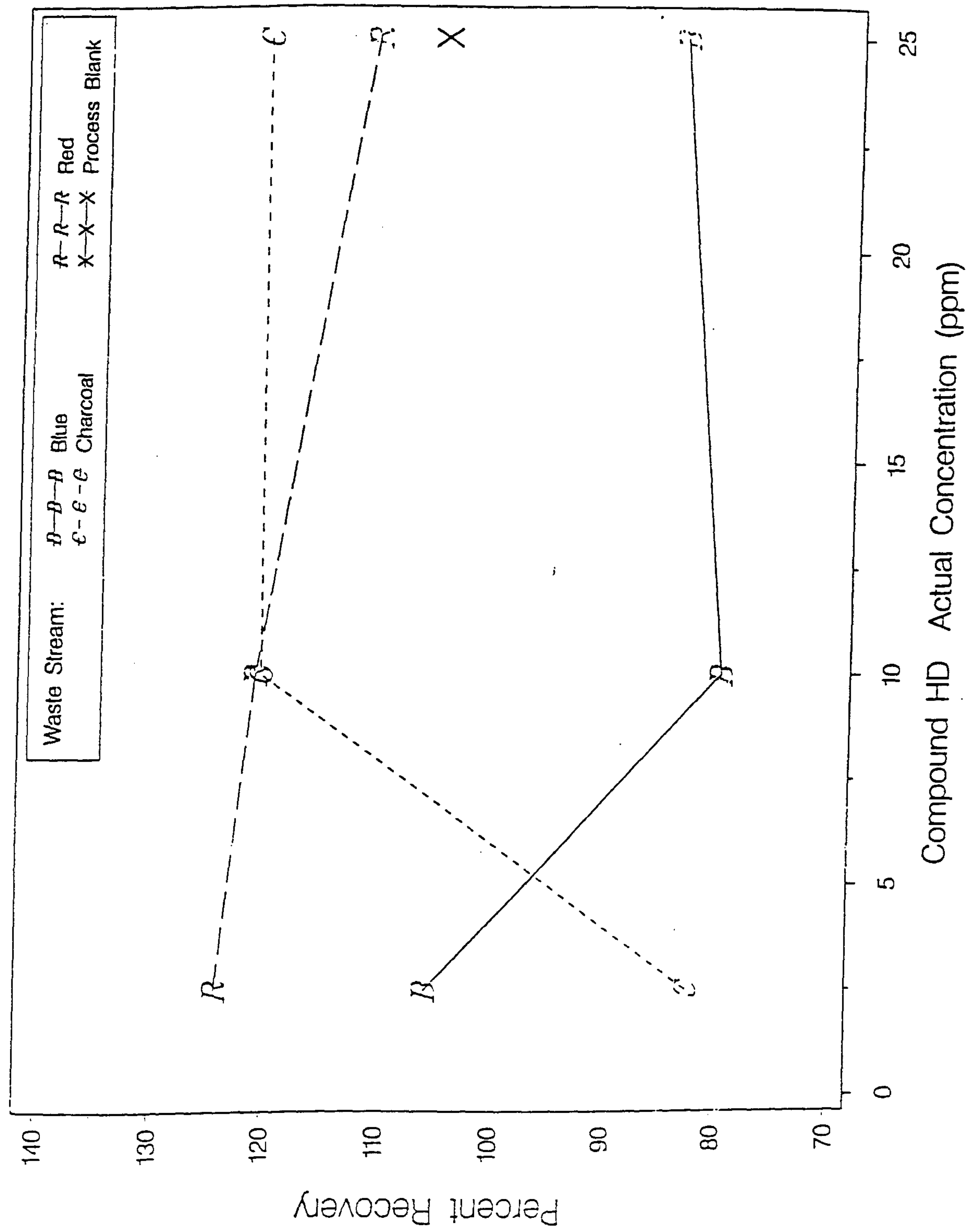


FIGURE 4. PERCENT RECOVERY OF HD FROM WASTESTREAM SAMPLES AVERAGED OVER THREE INJECTION DATES (EXCLUDING 7/10/96 FOR BLUE WASTESTREAM).

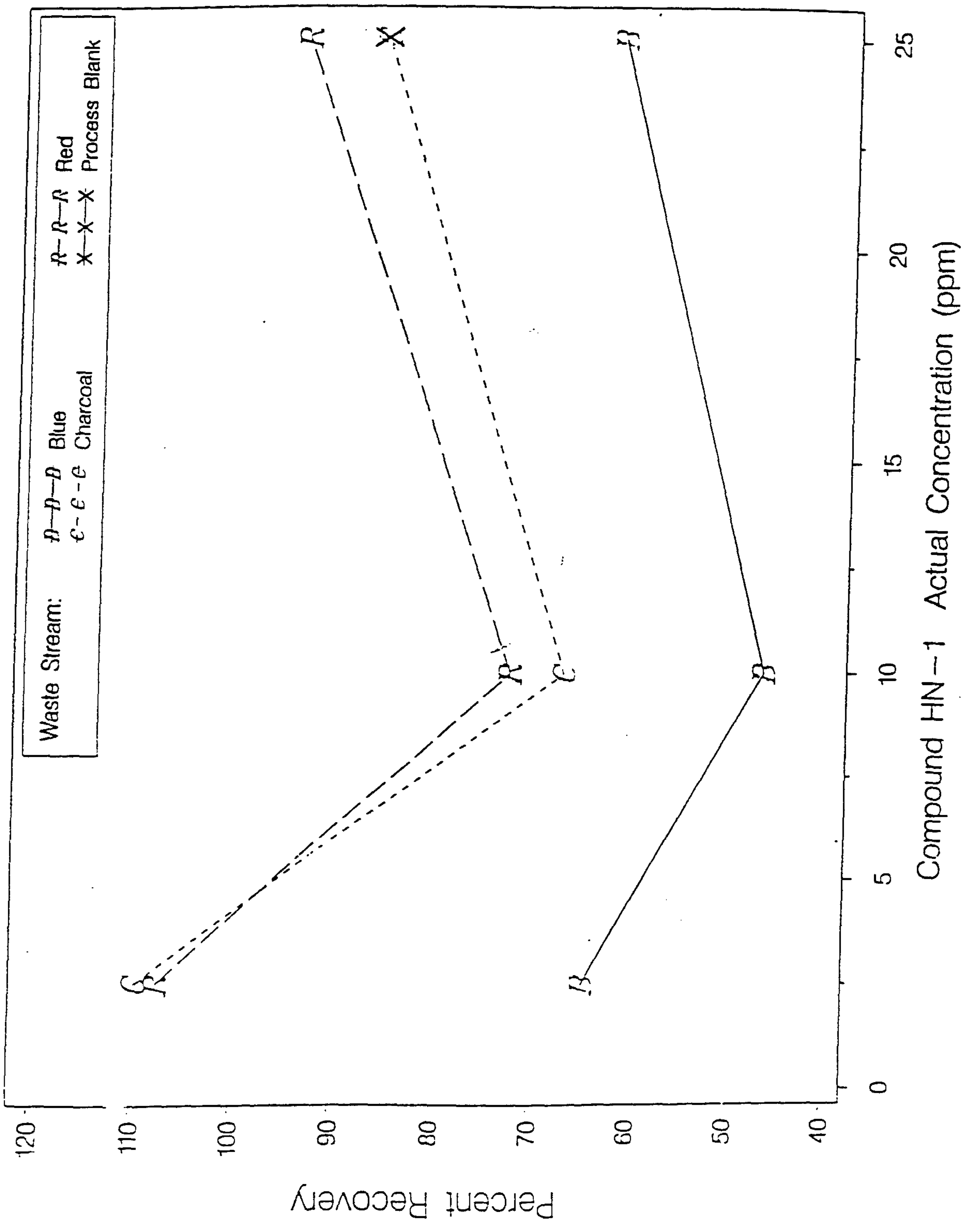


FIGURE 5. PERCENT RECOVERY OF HN-1 FROM WASTESTEAM SAMPLES AVERAGED OVER THREE INJECTION DATES (7/8-11/96).

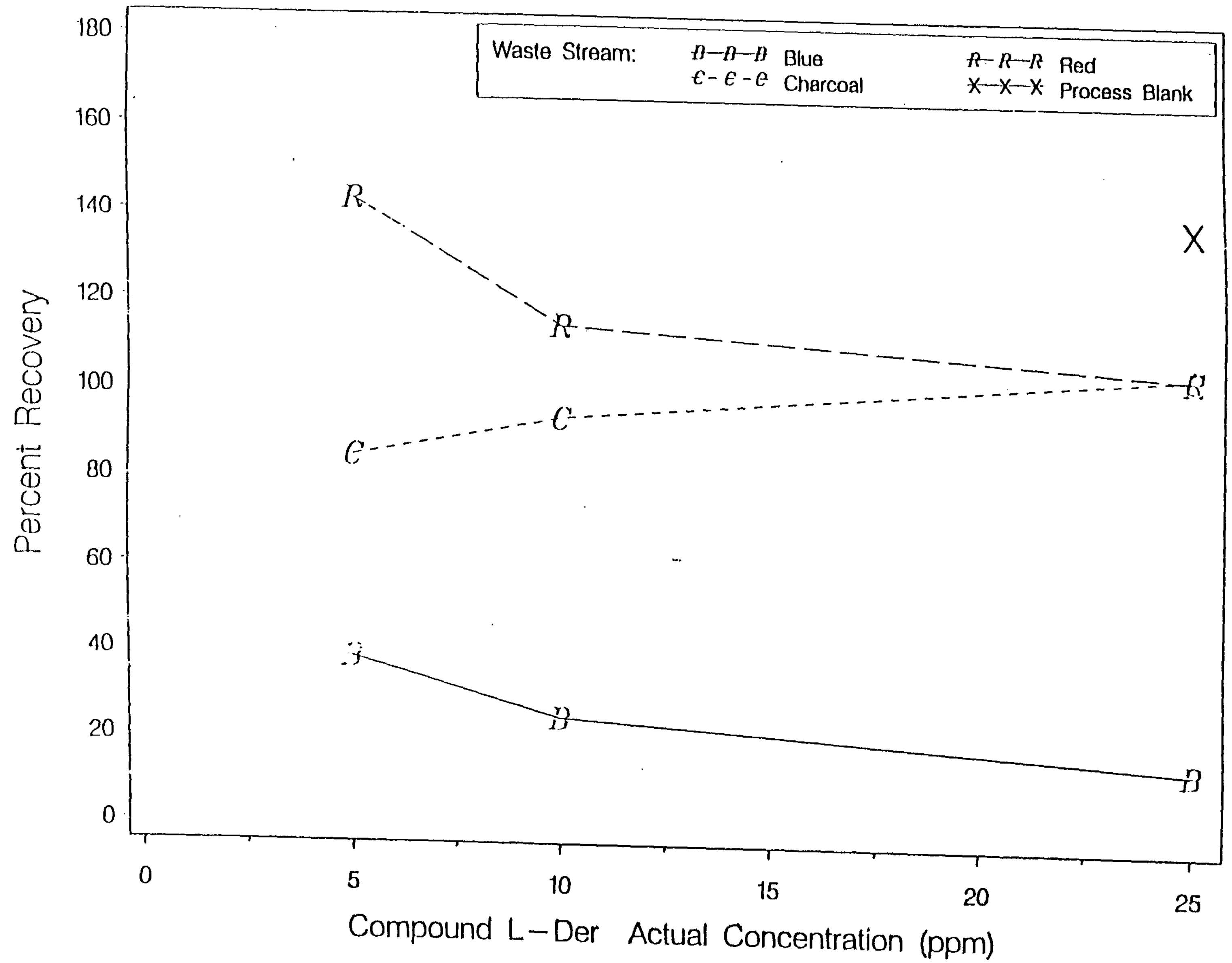


FIGURE 6. PERCENT RECOVERY OF L-Der FROM WASTESTREAM SAMPLES AVERAGED OVER THREE INJECTION DATES (7/8-11/96).

ATTACHMENT C

Analysis of Waste Streams

Task 95-38 Chemistry Report
G155538A
Summary of Waste Stream Analyses

On June 20 and 26, 1996, two waste streams (Blue Process June 17, 1996 Lot # 96-0037-057 and Red Process June 13, 1996 Lot # 96-0037-055) were analyzed by GC/MSD using the extraction procedure provided by the client on June 10, 1996. A low level of HD was found in the Blue sample and a low level of L was found in the Red sample. HN-1 was not detected in either sample, L was not detected in the Blue sample, and HD was not detected in the Red Sample. The results given in the tables were determined using regression parameters for calibration standards from 1 to 100 ppm based on a quadratic regression model. The data is recorded in LRB-356 and Task 38 Binder-02.

Table 1. Blue Waste Stream Analysis Results

Date	HD	L	HN-1
June 20	6.6 ppm	BDL	BDL
June 26	5.5 ppm	BDL	BDL
Statistics			
Average	6.1 ppm		
Std Dev	0.8 ppm		

Table 2. Red Waste Stream Analysis Results

Date	HD	L	HN-1
June 20	BDL	9.3 ppm	BDL
June 26	BDL	13.2 ppm	BDL
Statistics			
Average		11.3 ppm	
Std Dev		2.8 ppm	

Where BDL is below detection limit.

Table 3 summarizes the results for the analysis of RRS waste streams during August, 1996. The values given in the Table 3 represent the average for duplicate determinations using regression parameters for calibration standards from 1 to 100 ppm based on a quadratic regression model for L and simple linear regression models for HD and HN-1. The data are recorded in LRB-356 and Task 38 Binder-02.

Table 3. Waste Stream Analysis Results

Analysis and Dosing Date	Waste Stream[a]	HN-1	HD	L
August 13, 1996	Blue - 11/28/95	BDL[b]	16.2 ppm	BDL
August 13, 1996	Red - 11/28/95	BDL	BDL	3.8 ppm[c]
August 13, 1996	Charcoal - 1/25/96	BDL	BDL	7.8 ppm[d]
August 29, 1996	Charcoal - 950096-064	BDL	BDL	74.2 ppm

[a] The lot number or date received is used to identify the waste stream. [b] BDL is below the detection limit. [c] Value reported but it is below the method detection limit. [d] Value reported but the qualifiers were not satisfied and the value is below the method detection limit.

Since the qualifying ions were not satisfied for L in the Charcoal sample analyzed on August 13, full scan analyses were performed on the Charcoal - 950096-064 samples to verify the presence of L-Der. The mass spectra clearly show that the L-Der is present in the Charcoal - 950096-064 waste stream. The L value reported for Charcoal - 950096-064 (74.2 ppm) was higher than the value reported by Brian MacIver at ERDEC before shipment (10.7 ppm). This is the first analysis where the MREF measurement has been different than the value reported by ERDEC or by Battelle at Edgewood.

The relative oxidizing strength of each waste stream was determined as part of the analysis procedure. The oxidizing strength of the waste streams are compared to a fresh solution of DCDMH. The results for all of the waste streams are shown in Table 4.

Table 4. Relative Oxidizing Strength

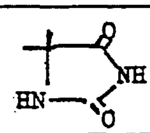
Waste Stream[a]	Analysis Date	% [Ox][b]
Charcoal - 950096-064	9/3/96	100%
Blue - 11/28/95	8/15/96	2%
Red - 11/28/95	8/15/96	7%
Charcoal- 1/25/96	8/15/96	3%
Red - 96-0037-047	6/21/96	25%
Red - 96-0037-055	6/21/96	33%
Blue - 96-0037-049	6/21/96	1%
Blue - 96-0037-057	6/21/96	1%
Charcoal - 95-0096-014L-018HN-1-023HD	6/21/96	5%
Charcoal- 1/25/96	6/21/96	3%

[a] The lot number or date received is used to identify the waste stream. [b] Relative oxidizing strength expressed in percent.

APPENDIX C

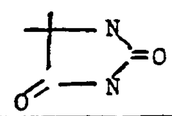
Studies Performed at ERDEC

PRODUCT ANALYSIS OF "ARCHIVED" WASTESTREAM[a]
("Blue" Process Chemistry)

Scan (sec)	Compound	Area % [b,c,d]
24	tertiary-butanol	22.8
27	chloroform	25.7
32	trichloroethene	0.1
35	CH_2Cl | CH_3-C-OH | CH_3	0.4
53	dichlorobutene	0.2
56	dichlorobutene	0.8
75	CH_2Cl | CH_3-C-OH | CH_2Cl	0.2
93	trichlorobutane	0.5
264	O || ClCH=CH-S-CH_2CH_2Cl	0.4
441		25.0
475	O || $ClCH_2CHCl$-S-CH_2CH_2Cl isomer	1.2
515	O || $ClCH_2CHCl$-S-CH_2CH_2Cl isomer	17.7
521	O || $ClCH_2CHCl$-S-CH $ClCH_2Cl$ isomer	3.5
558	O || $ClCH_2CHCl$-S-CH $ClCH_2Cl$ isomer	1.45

(a) Studies performed at ERDEC, composition analysis conducted via the GC-MS chemical ionization (CI) mode.
(b) Area % calculated from the Total Ion Chromatogram (TIC) of the mass spectrometer.
(c) Area % is semi-quantative, the intent is to show the percent of the peak in comparison to other peaks in the chromatogram. Peaks less than 0.1% of the TIC are not quantitated.
(d) Method Quantitation Limit (MQL) for agent is (50 ppm).

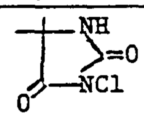
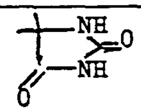
PRODUCT ANALYSIS OF "ARCHIVED" WASTESTREAM[a]
("Red" Process Chemistry)

Scan (sec)	Compound	Area % [b,c,d]
24	Tertiary-butanol	13.7
27	chloroform	46.7
32	OH / Cl_3CCH \ OH	1.5
36	CH_2Cl | CH_3C-OH | CH_3	7.2
40	Cl_2CHCH_2-OH	0.4
53	dichlorobutene + unknown (MW = 140)	0.3
56	unknown	1.3
69	trichlorobutane	0.4
75	CH_2Cl | CH_3C-OH | CH_2Cl	1.5
81	trichlorobutene	0.1
94	trichlorobutane	4.6
96	trichlorobutene	0.2
99	trichlorobutene	0.3
108	unknown (MW = 174)	0.05
136	trichlorobutene	1.4
148	unknown (MW = 208)	0.1
155	tetrachlorobutane	0.3
167	CH_2Cl | $ClCH_2$-C-OH isomer | CH_2Cl	2.1
188	scan 167 isomer	0.05
205	scan 167 isomer	0.2
220	scan 167 isomer	1.6
234	scan 167 isomer	0.2
292	tetrachlorobutene	0.2
328	unknown (MW = 216)	0.03
340	unknown (MW = 206)	0.06
407		15.2
518	unknown (MW = 242)	0.1
556	unknown	0.05
610	unknown	0.04

(a) Studies conducted at ERDEC, composition analysis conducted via the GC-MS chemical ionization (CI) mode
(b) Area % calculated from the Total Ion Chromatogram (TIC) of the mass spectrometer
(c) Area & is semi-quantative, the intent is to show the percent of the peak in comparison to other peaks in the chromatogram. Peaks less than 0.1% of the TIC are not quantitated.
(d) Method Quantitation Limit (MQL) for agent is (50 ppm).

Product Analysis of "Archived" Wastream[a]

("Charcoal" Process Chemistry)[a]

Scan (sec)	Compound	Area % [b,c,d]
37	OH / Cl_3CCH \\ OH	25.0
39	Dichlorobutane	17.5
60	Trichloroethylene	6.5
79	Dichlorobutyl alcohol	1.1
87	Trichlorobutene	1.5
98	Dichlorobutene	7.6
138	0 || $ClCH_2CH_2SCl$ || 0	4.4
141	Trichlorobutene	3.3
172	Trichlorobutene	3.4
205	CH_2CH_2Cl / HN \\ CH_2CH_2Cl	3.2
217	Unknown	4.6
225	Trichlorobutene	2.0
297	Tetrachlorobutene	0.6
369		Not quantitated
350-500		Not quantitated
483	Hexachlorobutene	15.9
523	0 || $Cl\text{-}CH_2CHCl\text{-}S\text{-}CHClCH_2Cl$ isomer	1.0
537	0 || $Cl\text{-}CH_2CH_2$ -S-$CHClCH_2Cl$ isomer || 0	1.7
566	0 || $Cl\text{-}CH_2CHCl\text{-}S\text{-}CHClCH_2Cl$ isomer || 0	0.4
579	0 || $Cl\text{-}CH_2CHCl\text{-}S\text{-}CHClCH_2Cl$ isomer || 0	0.2

(a) Studies performed at ERDEC, composition analysis conducted via the gc-ms chemical ionization (CI) mode
(b) Area % calculated from the Total Ion Chromatogram (TIC) of the mass spectrometer.
(c) Area % is semi-quantative, the intent is to show the percent of the peak in comparison to other peaks in the chromatogram. Peaks less than 0.1% of the TIC are not quantitated.
(d) Method Quantitation Limit (MQL) for agent is (50 ppm).

APPENDIX D

Gross Lesion Appearance (24-hr)

D-1

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A

Date: 2-20-96

MREF Protocol #: 109

Study Director: Carl Olson

Day: 2

Lesion Read By: CO

Lesions Recorded By: Dmm

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
301	15/10	15/8	16/14	13/14	22/23	19/22	23/20	readings taken in mm
	E-2 R-2	R-2 E-2	E-3 R-3	R-2 E-2	R-3 E-2	R-2 E-2	R-3 E-3	
Mean Average								

OWN 2-20-96 DMM

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 10ul 10% HD in $CHCl_3$

Site B 50ul 10% HD in $CHCl_3$

Site C 10ul 10% HN in $CHCl_3$

Site D 50ul 10% HN in $CHCl_3$

Site E 10ul 10% L in $CHCl_3$

Site F 50ul 10% L in $CHCl_3$

Site G 1ul neat HD

Reviewed By: C T Olson

Date: 2/23/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 2-20-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: CJO Lesions Recorded By: RMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
305	10/8	12/12	9/16	14/13	19/20	27/21	21/25	readings taken in mm
	R-2 E-2	R-3 E-3	R-2 E-2	R-2 E-2	R-2 E-2	R-3 E-3	R-2 E-2	
Mean Average								

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 10 ul 10% HN in $CHCl_3$

Site B 50 ul 10% HN in $CHCl_3$

Site C 10 ul 10% L in $CHCl_3$

Site D 50 ul 10% L in $CHCl_3$

Site E 10 ul 10% HD in $CHCl_3$

Site F 50 ul 10% HD in $CHCl_3$

Site G 1 ul neat HD

Reviewed By: [illegible] Date: 2/23/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 2-22-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: BH Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
306	15/8	7/8	7/8	16/17	16/13	12/14	12/10	readings taken in mm
	R-2 E-3	R-3 E-3	R-1 E-1	R-2 E-3	R-2 E-3	R-2 E-1	R-1 E-3	
Mean Average								

OIE 2-22-96 DMM

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5 ul 10% L in $CHCl_3$

Site B 10 ul 10% L in $CHCl_3$

Site C 5 ul 10% HD in $CHCl_3$

Site D 10 ul 10% HD in $CHCl_3$

Site E 5 ul 10% HN in $CHCl_3$

Site F 10 ul 10% HN in $CHCl_3$

Site G 1 ul neat HD

Reviewed By: C. T. Olson Date: 2/23/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A

Date: 2-22-96

MREF Protocol #: 109

Study Director: Carl Olson

Day: 2

Lesion Read By: BG

Lesions Recorded By: DMN

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
309	16/10	12/13	15/14	14/10	7/9	9/7	7/10	readings taken in mm
	R-2 E-2	R-2 E-2	R-3 E-3	R-3 E-2	R-3 E-3	R-2 E-2	R-3 E-3	
Mean Average								

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 10 ul 10% HD in $CHCl_3$

Site B 5 ul 10% HD in $CHCl_3$

Site C 10 ul 10% HN in $CHCl_3$

Site D 5 ul 10% HN in $CHCl_3$

Site E 10 ul 10% L in $CHCl_3$

Site F 5 ul 10% L in CHCl

Site G 1 ul neat HD

Reviewed By: C. T. Olson

Date: 2/23/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A

Date: 2-28-96

MREF Protocol #: 109

Study Director: Carl Olson

Day: 2

Lesion Read By: CTO

Lesions Recorded By: [illegible]

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
312	15/12	19/10	14/12	20/15	9/12	9/11	12/8	Readings taken in mm
	R-3 E-3	R-2 E-2	R-2 E-2	R-3 E-3	R-3 E-3	R-3 E-2	R-2 E-2	
Mean Average								

DWN 10-23-96 DMM

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 10ul 10% L in $CHCl_3$
Site B 5ul 10% L in $CHCl_3$
Site C 10ul 10% HD in $CHCl_3$
Site D 5ul 10% HD in $CHCl_3$
Site E 10ul 10% HN in $CHCl_3$
Site F 5ul 10% HN in $CHCl_3$
Site G 1ul neat HD

Reviewed By: C T Olson

Date: 2/28/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A

Date: 2-25-96

MREF Protocol #: 109

Study Director: Carl Olson

Day: 2

Lesion Read By: CTO

Lesions Recorded By: [illegible]

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
316	17/9	15/13	21/14	22/14	11/9	15/9	14/14	Readings taken in mm
	R-2 E-2	R-3 E-3	R-3 E-2	R-3 E-2	R-2 E-3	R-3 E 3	R-3 E-2	
Mean Average								

All Measurements in Millimeters.
N/A = Not applicable
N/R = Not required.

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Stie A 10 ul 10% HD in CHCl3
Site B 5 ul 10% HD in CHCl3
Site C 10 ul 10% L in CHCl3
Site D 5 ul 10% L in CHCl3
Site E 10 ul 10% HD in CHCl3
Site F 5 ul 10% HD in CHCl3
Site G 1 ul neat HD

Reviewed By: C T Olson

Date: 2/28/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-~~9001~~ 638A   Date: 3-6-96

MREF Protocol #: 109   Study Director: Carl Olson

Day: 2   Lesion Read By: CTO   Lesions Recorded By: JMH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #						④			
313	10/13	12/9	12/14	14/16	N/A / N/A	N/A / N/A	N/A / N/A	N/A / N/A	reading taken in mm
	R-2 E-1	R-2 E-3	R-3 E-2	R-3 E-3	~~R-0 E-0~~ ②JMH	R-0 E-1	R-0 E-1	R-0 E-1	
					R-0 E-1	②JMH			

②WJJ 3-6-96 JMH

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = Not apparent

④AC NA is equivalent to 0 on this form through out the study 10-24-96 DAC

Site A 5ul 10% HD in CHCl3

Site B 20ul neutralizing solution

Site C 5ul 10% HN in CHCl3

Site D 20ul neutralizing solution

Site E 5ul 10% L in CHCl3

Site F 20ul neutralizing solution

Site G 1ul neat HD

Site H 20ul neutralizing solution

Form No. MREF-LESION.SIZ-07

①Entry Error 3-6-96 JMH
③ E.E. 3-6-96 JMH

Reviewed by C T Olson
3/7/96

LESION SIZE DETERMINATION SHEET

Project #: ~~G1555-9001~~ 0384

Date: 3-6-96

MREF Protocol #: 109

Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: JMH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #					(2)				
315	15 / 9	12 / 13	11 / 9	18 / 12	N/A / N/A	N/A / N/A	N/A / N/A	N/A / N/A	reading taken in mm
	R-1 E-1	R-3 E-3	R-2 E-3	R-2 E-3	R-0 E-1	R-0 E-1	R-0 E-1	R-0 E-1	

0 = Not apparent OWN 3-6-96 DM

(2) AC NA is equivalent to 0 on this form throughout the study 10-24-96 DM

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5ul 10% HD in CHCl3

Site B 20ul neutralizing solution

Site C 5ul 10% L in CHCl3

Site D 20ul neutralizing solution

Site E 5ul 10% HD in CHCl3

Site F 20ul neutralizing solution

Site G 1ul neat HD

Site H 20ul neutralizing solution

Form No. MREF-LESION.SIZ-07

Reviewed by C J Olson 3/7/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-~~9001~~ 0334 Date: 3-6-96

MREF Protocol #: 109 Study Director: Carl Olson

Day: 2 Lesion Read By: CTO Lesions Recorded By: JMH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
317	9/15	8/8	12/12	15/10	0/0	0/0	0/0	0/0	reading taken JMH
	R-3 E-3	R-2 E-3	R-2 E-2	R-3 E-2	R-0 E-1	R-0 E-1	R-0 E-1	R-0 E-1	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = Not Apparent ① WN 3-6-96 DMA ② IE 3-6-96 DMA

Site A 5ul 10% L in CHCl3

Site B 20ul neutralizing solution

Site C 5ul 10% HD in CHCl3

Site D 20ul neutralizing solution

Site E 5ul 10% HN in CHCl3

Site F 20ul neutralizing solution

Site G 1ul neat HD

Site H 20 ul neutralizing solution

Form No. MREF-LESION.SIZ-07

Reviewed by C T Olson 3/7/96

LESION SIZE DE[illegible]MINATION SHEET

Project #: G1555-~~9001~~ 0354

Date: 3-6-96

MREF Protocol #: 109

Study Director: Carl Olson

Day: 2

Lesion Read By: CTO

Lesions Recorded By: JMH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
324	9/12	10/11	13/14	14/9	0/0	0/0	0/0	0/0	readings taken in mm
	R-3 E-3	R-2 E-2	R-3 E-3	R-2 E-3	R-0 E-0	R-0 E-1	R-0 E-1	R-0 E-1	

DWV 3-6-96 DRH

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = Not Apparent

Site A 5ul 10% HD in CHCl3

Site B 20ul neutralizing solution

Site C 5ul 10% HD in CHCl3

Site D 20ul neutralizing solution

Site E 5ul 10% L in CHCl3

Site F 20ul neutralizing solution

Site G 1ul neat HD

Site H 20ul neutralizing solution

Form No. MREF-LESION.SIZ-07

Reviewed by C T Olson 3/7/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-~~9001~~ 35.40

Date: 3-6-96

MREF Protocol #: 109

Study Director: Carl Olson

Day: 2

Lesion Read By: CTO

Lesions Recorded By: JMH

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
311	13/11	14/9	12/9	15/10	0/0	0/0	0/0	0/0	readings taken in mm
	R-2 E-2	R-3 E-3	R-3 E-2	R-2 E-2	R-0 E-1	R-0 E-1	R-0 E-1	R-0 E-1	

(1) WN 3-6-96 DMW
0= Not apparent (2) WN 3-6-96 DMW

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5ul 10% HN in CHCl3

Site B 20ul neutralizing solution

Site C 5ul 10% L in CHCl3

Site D 20ul neutralizing solution

Site E 5ul 10% HD in CHCl3

Site F 20ul neutralizing solution

Site G (2) 5ul neat HD

Site H 20ul neutralizing solution

Form No. MREF-LESION.SIZ-07

Appendix D

Reviewed by C T Olson
3/7/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A   Date: 3-14-96

MREF Protocol #: 109 Phase 3   Study Director: Carl Olson

Day: 2   Lesion Read By: AB   Lesions Recorded By: RMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
310	12/15	9/8	12/10	9/10	17/22	11/17	15/16	readings taken in mm
	R-1 E-2	R-1 E-2	R-3 E-3	R-3 E-3	R-1 E-2	R-1 E-2	R-0.5 (2) E-0.5	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

0 = Not apparent
R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

(1) EE 3-14-96 RMM
(2) AC The lesion readings were best determined at levels between (3) 0.0 and 1 and was therefore designated as 0.5. 3-14-96 RMM
(3) EE 10-16-96 RMM

Site A 5ul/10% HD in CHCl3
Site B 25ul Red Wastestream
Site C 5ul/10% HN in CHCl3
Site D 25ul Blue Wastestream
Site E 5ul/10% L in CHCl3
Site F 25ul Charcoal Wastestream
Site G 1ul neat HD

Reviewed by C T Olson
3/15/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
491	12/9	11/15	8/10	13/19	7/12	15/17	9/26	readings taken in mm
	R-3 E-3	R-3 E-2	R-1 ~~E-1~~	R-1 E-3	R 0.5④ E-3③	R-1 E-2	R-2① E-2	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

0 = Not apparent ① WN 3-14-96 DMM
R = Erythema ② AC 3-14-96 DMM
E = Edema ③ SE 3-14-96 DMM
1 = Mild
2 = Moderate
3 = Severe

Site A 5ul 10% L in CHCl3
Site B 25ul Charcoal Wastestream
Site C 5ul 10% HD in CHCl3
Site D 25ul Red Wastestream
Site E 5ul 10% HN in CHCl3
Site F 25ul Blue Wastestream
Site G 1ul neat HD

② Site A on day 2 appears to have a piece of skin pulled back across the lesion attached at one point. 3-14-96 DMM Could be due to ~~[illegible]~~ trauma induced over night by the animal. Elizabethan Collars were not used on any animals on this study day 3-14-96 DMM

④ AC The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5. 3-14-96 DMM

Form No. MREF-LESION.SIZ-07

Reviewed by CT Olson 3/15/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: AB Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
493	9/7	11/7	10/9	14/15	15/20	19/19	15/20	Readings taken in mm
	R-3 E-1	R-1 G-2	R-3 E-3	R-3 E-3	R-1 E-1	R-1 E-2	R-0.5 ① E-0.5	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

0 = not apparent
R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① AC The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5 3-14-96 DMM

Site A 5 ul 10% HD in $CHCl_3$

Site B 25 ul Red Wastestream

Site C 5 ul 10% HN in $CHCl_3$

Site D 25 ul Blue Wastestream

Site E 5 ul 10% L in $CHCl_3$

Site F 25 ul Charcoal Wastestream

Site G 1 ul neat HD

Form No. MREF-LESION.SIZ-07

Reviewed by C T Olson 3/18/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: CMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
498	7/9	12/11	9/10	17/15	17/23	11/20	17/19	Readings taken in mm
	R-3 E-2	R-3 E-3	R-3 E-3	R-2 E-3	R-3 E-1	R0.5 ① E0.5	R-1 E-2	

All measurements in millimeters
N/A = Not applicable
N/R = Not required

0=not apparent ① AC
R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5 3-14-96 Dmm

Site A 5ul 10% HN in $CHCl_3$

Site B 25ul Blue Wastestream

Site C 5ul 10% L in $CHCl_3$

Site D 25ul Charcoal Wastestream

Site E 5ul 10% HD in $CHCl_3$

Site F 25ul Red Wastestream

Site G 1 ul neat HD

Form No. MREF-LESION.SIZ-07

Appendix D

Reviewed by C T Olson
3/15/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-22-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: AB Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
499	7/8	10/10	11/11	12/16	13/15	15/16	9/16	
	R-2 E-2	R-2 E-3	R-3 E-1	R-3 E-2	R-2 E-1	R-0.5 E-0.5 (1)	R-1 E-2	
		U=4 (2)	U=6 (2)	U=4 (2)				

All measurements in millimeters
N/A = Not applicable
N/R = Not required

0 = not apparent
R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

(1) AC The lesion readings were best determinded at levels between 0.0 and 1 and was therefore designated as 0.5. 3-22-96 DMM

(2) AC ulceration of dose sites were not perviously recorded 3-28-96 DMM

U = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5 ul 10% HN in CHCl3
Site B 25 ul Blue Wastestream
Site C 5 ul 10% L in CHCl3
Site D 25 ul Charcoal Wastream
Site E 5 ul 10% HD in CHCl3
Site F 25 ul Red Wastestream
Site G 1 ul neat HD

Form No. MREF-LESION.SIZ-07

Reviewed by C.T. Olson
3/28/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-22-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: Dmm

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
494	14/13	10/13	10/11	17/15	0/0	21/19	13/20	
	R-3 E-3	R-2 E-2	R-2 E-2	R-3 E-3	R-0 E-0	R-1 E-1	R-2 E-1	
			U=4	U=4				

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = not apparent
OAC ulceration of dose sites w not previously recorded 3-28-96 Dmm
U = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5ul 10% L in CHCl3
Site B 25ul Charcoal Wastestream
Site C 5ul 10% HD in CHCl3
Site D 25ul Red Wastestream
Site E 5ul 10% HW in CHCl3
Site F 25ul Blue Wastestream
Site G 1ul neat HD

Form No. MREF-LESION.SIZ-07

Reviewed by C T Olson
3/25/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-22-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: Dmm

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
496	12/15	10/9	13/10	17/16	19/21	19/19	0/0	
	R-3 E-3	R-3 E-1	R-3 E-3	R-3 E-3	R-1 E-2	R-3 E-2	R-0 E-0	
	u=6 ①	u=6 ①	u=6 ①	u=4				

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = not apparent
① AC ulceration of dose sites not previously recorded 3-28-96 Dmm

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5ul 10% HD in CHCl3

Site B 25ul Red Wastestream

Site C 5ul 10% HW in CHCl3

Site D 25ul Blue Wastestream

Site E 5ul 10% L in CHCl3

Site F 25ul Charcoal Wastestream

Site G 1ul neat HD

Form No. MREF-LESION.SIZ-07

Reviewed by C I Olson
3/25/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 3-22-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: JB Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	COMMENTS
Animal I.D. #								
497	8/8	11/10	10/12	15/21	21/20	18/15	14/16	
	R-3 E-3	R-3 E-3	R-3 E-3	R-3 E-3	R-2 E-2	R-0.5 ② E-0.5 ②	R-2 E-2	
	U=6 ③		U=6 ③	U=6 ③				

OIE 3-22-96 DM

All measurements in millimeters
N/A = Not applicable
N/R = Not required

0 = not apparent
R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

② AC The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5 3-22-96 DMM

③ AC ulceration of dose sites were not previously recorded. 3-28-96 DMM

U = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5 ul 10% HN in CHCl3

Site B 25 ul Blue Wastestream

Site C 5 ul 10% L in CHCl3

Site D 25 ul Charcoal Wastestream

Site E 5 ul 10% HD in CHCl3

Site F 25 ul Red Wastestream

Site G 1 ul neat HD

Form No. MREF-LESION.SIZ-07

Appendix D

Reviewed by C.T. Olson
3/28/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-21-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: GK Lesions Recorded By: DPLM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
346	28/12	12/8	10/9	11/14	0/0	16/21	0/0	19/20	
	R-3 E-3	R-2 E-2	R-2 E-2	R-2 E-2	R-0 E-0	R-1 E-1	R-0 E-0	R-1 E-1	

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5 µl 10% L in $CHCl_3$

Site B 25 µl red montastream

Site C 5 µl 10% HD in $CHCl_3$

Site D 25 µl blue montastream

Site E 5 µl 10% HN in $CHCl_3$

Site F 25 µl red montastream

Site G 1 µl neat HD

Site H 25 µl blue montastream

Form No. MREF-LESION.SIZ-07

Appendix D

Reviewed by
C T Olson 6/24/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-21-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CH Lesions Recorded By: Dmm

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
341	11/12	11/8	14/13	15/9	24/13	0/0	11/12	0/0	
	R-2 E-2	R-2 E-2	R-3 E-3	R-3 E-3	R-2 E-2	R-0 E-0	R-3 E-2	R-0 E-0	
	u=5 ①	u=4 ①	u=6 ①				u=6 ①		

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① AC ulceration of dose sites were not previously recorded 6-21-96 Dmm ②
② WD 6-21-96 Dmm

Site A 5 µl 10% HD in $CHCl_3$

Site B 25 µl blue mustard

Site C 5 µl 10% HN in $CHCl_3$

Site D 25 µl red mustard

Site E 5 µl 10% L in $CHCl_3$

Site F 25 µl blue mustard

Site G 1 µl neat HD

Site H 25 µl red mustard

u = ulceration noted as:
4 = small
5 = medium
6 = large

Form No. MREF-LESION.SIZ-07

Reviewed by C T Olson 6/24/96

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-21-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BH Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
339	10/9	18/13	12/12	9/10	0/0	19/14	0/0	28/13	
	R-2 E-1	R-3 E-3	R-3 E-3	R-3 E-2	R-0 E-0	R-2 E-2	R-0 E-0	R-2 E-2	
		u=4 ①	u=4 ①						

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

①AC ulceration of dose sites were not previously recorded 6-21-96 DMM

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5 µl 10% HN in CHCl3

Site B 25 µl red mustastream

Site C 5 µl 10% L in CHCl3

Site D 25 µl blue mustastream

Site E 5 µl 10% HD in CHCl3

Site F 25 µl red mustastream

Site G 1 µl neat HD

Site H 25 µl blue mustastream

Reviewed by CT Olson 6/24/96

Form No. MREF-LESION.SIZ-07

Appendix D

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-21-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BH Lesions Recorded By: RMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
342	22/14	11/8	9/10	16/9	23/15	0/0	11/22	0/0	
	R-3 E-3	R-2 E-2	R-2 E-2	R-2 E-2	R-2 E-2	R-0 E-0	R-2 E-2	R-0 E-0	

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5 µl 10% L in $CHCl_3$

Site B 25 µl blue wastestream

Site C 5 µl 10% HD in $CHCl_3$

Site D 25 µl red wastestream

Site E 5 µl 10% HN in $CHCl_3$

Site F 25 µl blue wastestream

Site G 1 µl neat HD

Site H 25 µl red wastestream

Reviewed by
C T Olson 6/24/96

Form No. MREF-LESION.SIZ-07

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-27-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CH Lesions Recorded By: Dmm

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
340	12/11	8/8	11/14	11/14	19/12	19/14	22/16	0/0	
	R-2 E-2	R-2 E-2	R-3 E-3	R-2 E-2	R-2 E-1	R-1 E-1	R-2 E1	R-0 E-0	
	u=4 (1)			u=6 (1)					

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

(1) AC ulceration of dose sites were not previously record 6-27-96 Dmm

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5 ul 10% HD in CHCl3
Site B 25 ul blue wastestream
Site C 5 ul 10% HD in CHCl3
Site D 25 ul red wastestream
Site E 5 ul 10% L in CHCl3
Site F 25 ul blue wastestream
Site G 1 ul neat HD
Site H 25 ul red wastestream

Reviewed by C T Olson 6/25/96

Form No. MREF-LESION.SIZ-07

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-27-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BH Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
345	9/12	9/14	11/10	14/15	0/0	19/15	21/15	20/16	
	R-3 E-3	R-3 E-3	R-2 E-3	R-2 E-3	R-0 E-0	R-2 E-1	R-1 E-1	R-2 E-1	
	u=6 1	u=6 1	u=6 1	u=6 1				u=4	

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

1 A C ulceration of dose sites were not previously recorded 6-27-96

Site A 5 ul 10% L in CHCl3

Site B 25 ul red Wastestream

Site C 5 ul 10% HD in CHCl3

Site D 25 ul blue Wastestream

Site E 5 ul 10% HD in CHCl3

Site F 25 ul red Wastestream

Site G 1 ul neat HD

Site H 25 ul blue Wastestream

u = ulceration noted as:
4 = small
5 = medium
6 = large

Reviewed by C T Olson 6/25/96

Form No. MREF-LESION.SIZ-07

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-27-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: 61 Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
351	13/15	15/9	10/15	15/14	16/19	15/12	21/12	0/0	
	R-2 E-2	R-3 E-3	R-3 E-2	R-3 E-2	R-1 E-2	R-1 E-1	R-1 E-2	R-0 E-0	
		u-4 ①	u-5 ①	u-5 ①					

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

① AC ulceration of dose sites were not previously recorded. 6-27-96 DMM

u = ulceration noted as:
4 = small
5 = medium
6 = large

Site A 5ul 10% HN in CHCl3
Site B 25ul blue Wastestream
Site C 5ul 10% L in CHCl3
Site D 25ul red Wastestream
Site E 5ul 10% HD in CHCl3
Site F 25ul blue Wastestream
Site G 1ul neat HD
Site H 25ul red Wastestream

Reviewed by C T Olson 6/25/96

Form No. MREF-LESION.SIZ-07

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 6-27-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: BH Lesions Recorded By: DMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
352	11/9	12/9	11/12	13/15	0/0	16/15	15/12	15/9	
	R-2 E-2	R-1 E-~~1~~2	R-3 E-3	R-3 E-3	R-0 E-0	R-1 E-2	R-1 E-1	R-2 E-1	
	u-5 ②	u-4 ②		u-6 ②					

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5ul 10% HD in CHCl3
Site B 25ul red Wastestream
Site C 5ul 10% HN in CHCl3
Site D 25ul blue Wastestream
Site E 5ul 10% L in CHCl3
Site F 25ul red Wastestream
Site G 1ul neat HD
Site H 25ul blue Wastestream

① WN 6-27-96 DMM

② AC ulceration of dose sites were not previously recorded 6-27-96 DMM

u = ulceration noted as:
4 = small
5 = medium
6 = large

Reviewed by C T Olson 6/28/96

Form No. MREF-LESION.SIZ-07

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: Dxm

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
383	15/20	11/18	12/18	①	6/21	10/11	16/14	①	
	R-3 E-3	R-2 E-2	R-2 E-2		R-1 E-1	R-.5② E-0	R-1 E-1		

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 10ul 10% L in CHCl3
Site B 10ul blue wastestream
Site C 10ul 10% HD in CHCl3
Site D 10ul Charcoal wastestream
Site E 10ul 10% HN in CHCl3
Site F 10ul red wastestream
① ~~Site G~~
① ~~Site H~~

Reviewed by C.T. Olson 8/14/96

Form No. MREF-LESION.SIZ-07

① AC These sites were not used on ③ ~~this day~~ 8-13-96 Dxm

② AC The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5 8-14-96 Dxm

③ WC 8-14-96 Dxm

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: AB Lesions Recorded By: ACmm

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
385	13/15	13/18	12/16	①	12/12	15/17	11/9	①	
	R-2 E-2	R-3 E-2	R-1 E-2		R-2 E-2	R-1 E-1	R-.5③ E-0		

0 = ② ~~was~~ not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 10ul 10% HN in CHCl3
Site B 10ul red Wastestream
Site C 10ul 10% L in CHCl3
Site D 10ul blue Wastestream
Site E 10ul 10% HD in CHCl3
Site F 10ul Charcoal Wastestream.

Reviewed by CTOF 8/14/96

① ~~Site G~~

① ~~Site H~~

(APPENDIX D)
Form No. MREF-LESION.SIZ-07

① AC These sites were not used on ~~this day~~ Ⓔ 8-13-96 Amm
② IE 8-14-96 Amm.
③ AC The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5 8-14-96 Amm

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: GMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
400	17/17	15/15	15/21	0	5/9	11/14	12/14	0	
	R-3 E-3	R-3 E-3	R-3 E-3		R-.5 (2) E-0	R-1 E-0	R-1 E-1		

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 10ul 10% HD in CHCl3

Site B 10ul Charcoal Wastestream

Site C 10ul 10% HN in CHCl3

Site D 10ul red wastestream

Site E 10ul 10% L in CHCl3

Site F 10ul blue Wastestream

(1) ~~Site G~~

(1) ~~Site H~~

Reviewed by C.T. Olson 8/14/96

(Appendix D)

Form No. MREF-LESION.SIZ-07

(1) AC These sites were not used on ~~this day~~ (3) 8-13-96 GMM

(2) AC The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5 8-14-96 GMM

(3) WIC 8-14-96 GMM

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-14-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: [illegible] Lesions Recorded By: EMM

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
389	13/13	12/21	11/21	1	5/19	11/16	11/9	1	
	R-3 E-2	R-3 E-3	R-3 E-2		R-.5 2 E-0	R-1 E-1	R-0 E-0		

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 10 ul 10% HL in CHCl3
Site B 10 ul red wastestream
Site C 10 ul 10% L in CHCl3
Site D 10 ul blue wastestream
Site E 10 ul 10% HD in CHCl3
Site F 10 ul charcoal wastestream

Reviewed by C.T. Olson 8/14/96

1 ~~Site G~~

1 ~~Site H~~

(Appendix D)
Form No. MREF-LESION.SIZ-07

1 AC These sites were not used on 3 ~~this day~~ 8-17-96 EMM
2 AC The lesion readings were best determined at levels between 0.0 and 1 and was therefore designated as 0.5 8-14-96 EMM
3 WW 8-14-96 EMM

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A   Date: 8-30-96

MREF Protocol #: 109 Phase 3   Study Director: Carl Olson

Day: 2   Lesion Read By: CTO   Lesions Recorded By: Rec

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
379	14/9	13/10	10/14	①	0/0	0/0	0/0	①	
	R-3 E-3	R-3 E-3	R-1 E-1		R-0 E-0	R-0 E-0	R-0 E-0		

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

0 = not apparent

Site A 5ul 10% L in CHCl3
Site B 25ul Charcoal Wastestream
Site C 5ul 10% HD in CHCl3
Site D 25ul Charcoal Wastestream
Site E 5ul 10% HV in CHCl3
Site F 25ul Charcoal Wastestream
① ~~Site G~~
① ~~Site H~~

Reviewed by C. T. Olson 8/30/96

Form No. MREF-LESION.SIZ-07

① AC These sites were not used on 8-29-96. Rec

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-30-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CTO Lesions Recorded By: RCL

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
380	10/10	12/10	8/8	①	0/0	0/0	0/0	①	
	R-1 E-2	R-3 E-2 ⓔ	R-3 E-3		R-0 E-0	R-0 E-0	R-0 E-0		

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

ⓔ RCL 1x2mm ulceration 8-30-96

0 = not apparent.

Site A 5ul 10% HN in CHCl3

Site B 25ul charcoal wastestream

Site C 5ul 10% L in CHCl3

Site D 25ul charcoal wastestream

Site E 5ul 10% HD in CHCl3

Site F 25ul charcoal wastestream

① ~~Site G~~

① ~~Site H~~

Reviewed by C T Olson 8/30/96

Form No. MREF-LESION.SIZ-07

① AC These sites were not used on 8-29-96. RCL

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-30-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CTO Lesions Recorded By: Per

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
387	11/12	10/9	16/13	∅	0/0	0/0	0/0	∅	
	R-3 E-3	R-2 E-2	R-3+ E-3+		R-0 E-0	R-0 E-0	R-0 E-0		

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5 ul 10% HD in CHCl3

Site B 25 ul charcoal wastestream

Site C 5 ul 10% HD in CHCl3

Site D 25 ul charcoal wastestream

Site E 5 ul 10% L in CHCl3

Site F 25 ul charcoal wastestream

∅ ~~Site G~~ ____________

∅ ~~Site H~~ ____________

Reviewed by C T Olson 8/30/96

Form No. MREF-LESION.SIZ-07

∅ These sites were not used on 8-29-96. Per

LESION SIZE DETERMINATION SHEET

Project #: G1555-38A Date: 8-30-96

MREF Protocol #: 109 Phase 3 Study Director: Carl Olson

Day: 2 Lesion Read By: CTO Lesions Recorded By: Rek

Lesion Sites	A	C	E	G	B	D	F	H	COMMENTS
Animal I.D. #									
388	14/9	13/10	15/9	①	0/0	0/0	0/0	①	
	R-3 E-3	R-3 E-3	R-2 E-3		R-0 E-0	R-0 E-0	R-0 E-0		
				Rek				Rek	

0 = not apparent

All measurements in millimeters
N/A = Not applicable
N/R = Not required

R = Erythema
E = Edema
1 = Mild
2 = Moderate
3 = Severe

Site A 5ul 10% L in $CHCl_3$

Site B 25ul Charcoal Wastestream

Site C 5ul 10% HD in $CHCl_3$

Site D 25ul Charcoal Wastestream

Site E 5ul 10% HN in $CHCl_3$

Site F 25ul Charcoal Wastestream

① ~~Site G~~

① ~~Site H~~

Reviewed by C T Olson 8/30/96 CTO

WD CTO 8/30/96

Form No. MREF-LESION.SIZ-07

① AC These sites were not used on 8-29-96. Rek

APPENDIX E

Dosage Site Code and Histopathology

Definitions Used in Histopathologic Evaluations and an Explanation of the Grading of Lesion Severity

Microblister: Loss of epidermal basal cell attachment to the underlying basement membrane of at least two adjacent cells. The loss of attachment creates a space which may appear empty, full of proteinaceous fluid, or filled with neutrophils. One or a few isolated small areas of detachment is graded 1, minimal. Many such areas of detachment, or several larger (10 or more contiguous cells) areas of detachment is graded 2, mild. When half or more of the epidermis in the tissue section is detached from the dermis, it is graded 3, moderate. Such lesions typically have a much larger space between the basal cells and the dermis. When nearly all of the epidermis is separated from the dermis, it is graded 4, marked. In such situations, there are usually focal, point attachments, so the entire epidermis is not lifted along the full width of the section.

Epidermal necrosis: The epidermal cells exhibit cytoplasmic eosinophilia, nuclear loss or pyknosis, and are generally shrunken. If only individual cells are affected, it is graded 1 (these are generally isolated basal cells). If small areas are affected, with normal areas in close proximity, it is graded 2. If the epidermis exhibits cell death in a full-thickness (all layers of epidermis) pattern, and affects half or more of the skin section, it is graded 3. If the epidermis is virtually entirely necrotic, it is graded 4. Severe ulcers assume that the epidermis is necrotic.

Follicular necrosis: If isolated epithelial cells of the hair follicles exhibit eosinophilia or pyknosis, it is graded 1. If clusters of adjacent cells within follicles are dead, it is graded 2. If cells of half or more of a particular hair follicle are dead, it is graded 3. Grade 4 lesions have complete necrosis of the follicular epithelium underlying much of the epidermal lesion area. This indicates that the agent has penetrated deeply.

Dermal necrosis: Loss of collagen fiber integrity, evidenced by pale eosinophilic staining and homogeneous appearance, indicates necrosis of dermal fibers. With only isolated areas, it is graded 1. Multiple areas are graded 2. Necrosis of most of the superficial dermal collagen in the lesion area is graded 3. A grade four lesion requires deep (to the base of the associated adnexa) dermal necrosis.

Hemorrhage: Extravasated erythrocytes is hemorrhage. A few isolated foci is graded 1. Multiple, common foci is graded 2. Large pools of blood is graded 3. A grade four lesion requires a massive area of blood pooling with displacement of large areas of dermal collagen.

Vascular necrosis: Loss of integrity of a medium to large blood vessel is vascular necrosis. Grading depends upon the number of vessels affected and the severity. Partial necrosis of one vessel is graded 1 to 2. Complete necrosis of a vessel is graded 3; multiple such lesions are graded 4.

Pustular epidermitis: Collections of neutrophils in the epidermis proper is graded by extent; one or two small foci is graded 1; three or more small foci is graded 2; one or more large foci is graded 3; a grade four lesion would indicate massive infiltration of the entire epidermis by neutrophils.

Task 95-38, Phase 2a, Day 1

Key for HGPs #301 and 305 dosed 2/19/1996. Exposure duration - 2 hr.

Animal # 301

Site	Treatment
A	10 μL of 10% HD in $CHCl_3$
B	50 μL of 10% HD in $CHCl_3$
C	10 μL of 10% HN in $CHCl_3$
D	50 μL of 10% HN in $CHCl_3$
E	10 μL of 10% L in $CHCl_3$
F	50 μL of 10% L in $CHCl_3$
G	1 μL of neat HD
H	

Animal #305

Site	Treatment
A	10 μL of 10% HN in $CHCl_3$
B	50 μL of 10% HN in $CHCl_3$
C	10 μL of 10% L in $CHCl_3$
D	50 μL of 10% L in $CHCl_3$
E	10 μL of 10% HD in $CHCl_3$
F	50 μL of 10% HD in $CHCl_3$
G	1 μL of neat HD
H	

Dosing Date: 2/19/96

MREF Task 95-38
G1555-38A

Animal # 301	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		2	2	2	2	3	4	2
Epidermal Necrosis		2	4	4	3	3	4	3
Follicular Necrosis		3	4	4	4	2	4	4
Dermal Necrosis		0	0	0	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	1	2	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: all lesions are centrally located; some normal skin present on all			mild dermal inflam	min dermal inflam	min dermal inflam	mild dermal inflam	mild dermal inflam	min dermal inflam

Animal # 305	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		2	2	3	3	3	2	2
Epidermal Necrosis		4	4	4	4	4	4	4
Follicular Necrosis		3	4	4	4	4	4	4
Dermal Necrosis		0	0	0	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	1	0	1	1	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: all lesions are centrally located; some normal skin present on all		mild dermal inflam	mild dermal inflam	mild dermal inflam		mild dermal inflam	min dermal inflam	mild dermal inflam

Degree of Severity Grading Scale:
0 = Normal, 1 = Minimal, 2 = Intermediate, 3 = Moderate, 4 = Severe
A. W. Singer, DVM, DACVP

Task 95-38, Phase 2a, Day 2

Key for HGPs #306 and 309 dosed 2/21/1996. Exposure duration - 2 hr.

Animal # 306

Site	Treatment
A	5 μL of 10% L in $CHCl_3$
B	10 μL of 10% L in $CHCl_3$
C	5 μL of 10% HD in $CHCl_3$
D	10 μL of 10% HD in $CHCl_3$
E	5 μL of 10% HN in $CHCl_3$
F	10 μL of 10% HN in $CHCl_3$
G	1 μL of neat HD
H	

Animal #309

Site	Treatment
A	10 μL of 10% HD in $CHCl_3$
B	5 μL of 10% HD in $CHCl_3$
C	10 μL of 10% HN in $CHCl_3$
D	5 μL of 10% HN in $CHCl_3$
E	10 μL of 10% L in $CHCl_3$
F	5 μL of 10% L in $CHCl_3$
G	1 μL of neat HD
H	

Animal # 306	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		4	3	1***	3	4	2***	1***
Epidermal Necrosis		4	4*	4***	4	4	4***	4***
Follicular Necrosis		4	4	4	4	4	4	4
Dermal Necrosis		1	1**	2	0	0	2	2
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		1	0	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: *focal ulceration **deep dermal edema ***large ulcer precludes much blister potential		mod dermal inflam	mild dermal inflam	mild dermal inflam	min dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam

Animal # 309	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		3	0*	4	4	4	4	3
Epidermal Necrosis		3	4*	4	4	4	4	4
Follicular Necrosis		4	4	3	2	4	3	4
Dermal Necrosis		1	2	0	0	0**	0	0
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	1	0	0
Pustular Epidermitis		1	0	1	1	0	0	0
Notes: *large ulceration precludes blister potential **deep dermal edema		mild dermal inflam	mild dermal inflam	mod dermal inflam	mod derm infla m	mod dermal inflam	mod dermal inflam	mild derm infla m

Note: Some normal skin is present on all sections, both animals; lesions are centrally located in trimmed area.

Degree of Severity Grading Scale:
0 = Normal; 1 = Minimal; 2 = Mild; 3 = Moderate; 4 = Severe   Allen W. Singer, D.V.M.

Task 95-38, Phase 2a, Day 3

Key for HGPs #312 and 316 dosed 2/27/1996. Exposure duration - 1 hr.

`Animal # 312

Site	Treatment
A	10 μL of 10% L in $CHCl_3$
B	5 μL of 10% L in $CHCl_3$
C	10 μL of 10% HD in $CHCl_3$
D	5 μL of 10% HD in $CHCl_3$
E	10 μL of 10% HN in $CHCl_3$
F	5 μL of 10% HN in $CHCl_3$
G	1 μL of neat HD
H	

Animal #316

Site:	Treatment
A	10 μL of 10% HN in $CHCl_3$
B	5 μL of 10% HN in $CHCl_3$
C	10 μL of 10% L in $CHCl_3$
D	5 μL of 10% L in $CHCl_3$
E	10 μL of 10% HD in $CHCl_3$
F	5 μL of 10% HD in $CHCl_3$
G	1 μL of neat HD
H	

Animal # 312	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		3	3	3	3	4	3	3
Epidermal Necrosis		4	4	4	4	4	4	4
Follicular Necrosis		4	4	4	4	4	3	4
Dermal Necrosis		0*	0*	0	0**	0	0	0*
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		1	2	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	1	2	0
Notes: *mod dermal edema **minimal dermal edema		mild dermal inflam	mod dermal inflam	mild dermal inflam	mild derm infla m	mild dermal inflam	mod derm inflam	mild derm infla m

Animal # 316	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		3	4	4	4	3	3	3
Epidermal Necrosis		4	4	4	4	4	4	4
Follicular Necrosis		4	3	4	4	4	4	4
Dermal Necrosis		0*	0	0**	0**	0	1	0**
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	2	2	1	0	0
Pustular Epidermitis		1	1	0	0	1	1	2
Notes: *minimal dermal edema **moderate dermal edema		mod dermal inflam	mod dermal inflam	mod dermal inflam	sever e derm infla m	mild dermal inflam	mod dermal inflam	mod derm infla m

Note: All sections (312 and 316) have normal, unaffected skin at one or both margins of the section.

Degree of Severity Grading Scale:
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

Allen W. Singer, D.V.M.

Task 95-38, Phase 2b, Day 1

Key for HGPs #311, 313, 315, 317, and 324 dosed 3/5/1996. Exposure duration - 1 hr.

Animal # 311

Site	Treatment
A	5 μL of 10% HN in $CHCl_3$
B	20 μL of neutralization solution
C	5 μL of 10% L in $CHCl_3$
D	20 μL of neutralization solution
E	5 μL of 10% HD in $CHCl_3$
F	20 μL of neutralization solution
G	1 μL of neat HD
H	20 μL of neutralization solution

Animal # 313

Site	Treatment
A	5 μL of 10% HD in $CHCl_3$
B	20 μL of neutralization solution
C	5 μL of 10% HN in $CHCl_3$
D	20 μL of neutralization solution
E	5 μL of 10% L in $CHCl_3$
F	20 μL of neutralization solution
G	1 μL of neat HD
H	20 μL of neutralization solution

Animal # 315

Site	Treatment
A	5 µL of 10% HN in $CHCl_3$
B	20 µL of neutralization solution
C	5 µL of 10% L in $CHCl_3$
D	20 µL of neutralization solution
E	5 µL of 10% HD in $CHCl_3$
F	20 µL of neutralization solution
G	1 µL of neat HD
H	20 µL of neutralization solution

Animal # 317

Site	Treatment
A	5 µL of 10% L in $CHCl_3$
B	20 µL of neutralization solution
C	5 µL of 10% HD in $CHCl_3$
D	20 µL of neutralization solution
E	5 µL of 10% HN in $CHCl_3$
F	20 µL of neutralization solution
G	1 µL of neat HD
H	20 µL of neutralization solution

Animal # 324

Site	Treatment
A	5 μL of 10% HD in $CHCl_3$
B	20 μL of neutralization solution
C	5 μL of 10% HN in $CHCl_3$
D	20 μL of neutralization solution
E	5 μL of 10% L in $CHCl_3$
F	20 μL of neutralization solution
G	1 μL of neat HD
H	20 μL of neutralization solution

Animal # 311	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		3	0	3	0	2	0	3	0
Epidermal Necrosis		4	0	4	0	4	0	4	0
Follicular Necrosis		2	0	4	0	4	0	4	0
Dermal Necrosis		0	0	0	0	0	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		2	0	3	0	0	0	1	0
Pustular Epidermitis		2	0	0	0	1	0	0	0
Note: *moderate deep dermal edema		mod dermal inflam		mod dermal inflam		mod dermal inflam		mod derm infla m	

Animal # 313	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		3	0	4	0	4	0	2	0
Epidermal Necrosis		4	0	4	0	4	0	4	0
Follicular Necrosis		4	0	4	0	3	0	4	0
Dermal Necrosis		0	0	1	0	0	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	1	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0	0
Note: *moderate deep dermal edema		mild derm inflam		mod dermal inflam	min dermal inflam	mod dermal inflam		mild dermal inflam	

Animal # 315	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		2	0	4	0	3	0	2	0
Epidermal Necrosis		3	0	4	0	4	0	4	0
Follicular Necrosis		2	0	4	0	4	0	4	0
Dermal Necrosis		0	0	1	0	1	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	1	0	2	0	0	0
Pustular Epidermitis		1	0	0	0	0	0	0	0
Note: *moderal dermal edema		mod dermal inflam		marked dermal inflam		mod dermal inflam		mild dermal inflam	

Animal # 317	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		2	0	2	0	3	0	2	0
Epidermal Necrosis		4	0	4	0	4	0	4	0
Follicular Necrosis		4	0	4	0	3	0	4	0
Dermal Necrosis		0*	0	2**	0	0	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		2	0	1	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	2	0	0	0
Notes: *mild dermal edema **focal ulceration(s)		mild dermal inflam	min dermal inflam	mod dermal inflam		mod dermal inflam		mild dermal inflam	

Animal # 324	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		4	0	4	0	4	0	3	0
Epidermal Necrosis		4	0	4	0	4	0	4	0
Follicular Necrosis		4	0	2	0	4	0	4	0
Dermal Necrosis		1	0	0	0	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	1	0	0	0
Pustular Epidermitis		0	0	1	0	0	0	0	0
Notes:		mod dermal inflam	min dermal inflam	mod dermal inflam		mod dermal inflam		min dermal inflam	

Note: Normal (unaffected) skin present laterally on all sections where lesions were observed.

Histopathological Markers 3/7/96
Degree of Severity Grading Scale Allen W. Singer, DVM

0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

Task 95-38, Phase 3, Day 1

Key for HGPs #310, 491, 493, and 498 dosed 3/13/1996. Exposure duration - 1 hr.

Animal # 310

Site	Treatment
A	5 µL of 10% HD in $CHCl_3$
B	25 µL of Red waste stream
C	5 µL of 10% HN in $CHCl_3$
D	25 µL of Blue waste stream
E	5 µL of 10% L in $CHCl_3$
F	25 µL of Charcoal waste stream
G	1 µL of neat HD
H	

Animal # 491

Site	Treatment
A	5 µL of 10% L in $CHCl_3$
B	25 µL of Charcoal waste stream
C	5 µL of 10% HD in $CHCl_3$
D	25 µL of Red waste stream
E	5 µL of 10% HN in $CHCl_3$
F	25 µL of Blue waste stream
G	1 µL of neat HD
H	

Animal # 493

Site	Treatment
A	5 µL of 10% HD in $CHCl_3$
B	25 µL of Red waste stream
C	5 µL of 10% HN in $CHCl_3$
D	25 µL of Blue waste stream
E	5 µL of 10% L in $CHCl_3$
F	25 µL of Charcoal waste stream
G	1 µL of neat HD
H	

Animal # 498

Site	Treatment
A	5 µL of 10% HN in $CHCl_3$
B	25 µL of Blue waste stream
C	5 µL of 10% L in $CHCl_3$
D	25 µL of Charcoal waste stream
E	5 µL of 10% HD in $CHCl_3$
F	25 µL of Red waste stream
G	1 µL of neat HD
H	

Animal # 310	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		2	0	4	2	1	0	1
Epidermal Necrosis		4	1	4	4	4*	2	4*
Follicular Necrosis		4	0	4	1	4	0	4
Dermal Necrosis		0	0	1	0	3	0	3**
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	1
Pustular Epidermitis		0	1	0	1	0	1	0
Notes: *marked ulceration **moderate dermal edema		mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mild dermal inflam	mod dermal inflam

Animal # 491	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		4	0	1	0	4	3	2
Epidermal Necrosis		4*	1	4**	0	4	4	4
Follicular Necrosis		4	0	4	0	3	0	4
Dermal Necrosis		3	0	3	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		2	0	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: *mild ulceration **marked ulceration		mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam

Animal # 493	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		1*	0	4	4	2	0	2*
Epidermal Necrosis		4**	0	4	4	4**	1	4**
Follicular Necrosis		4	0	3	0	4	0	4
Dermal Necrosis		3	0	0	0	3	0	3
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	1	0	0
Pustular Epidermitis		0	1	0	0	0	0	0
Notes: *at edge of ulcer **marked ulceration		mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam

Animal # 498	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		2*	3	3	0	3	0	1
Epidermal Necrosis		4**	4***	4***	0	4**	0	4***
Follicular Necrosis		4	0	4	0	4	0	4
Dermal Necrosis		3	1	2	0	3	0	2
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	1	0	0	0	0
Pustular Epidermitis		1	0	0	1	1	0	0
Notes: *at edge of ulcer **marked ulceration ***minimal ulceration		mod dermal inflam	mild dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mild dermal inflam	mild dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale 3/18/96
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe Allen W. Singer, DVM

Task 95-38, Phase 3, Day 2

Key for HGPs #494, 496, 497, and 499 dosed 3/21/1996. Exposure duration - 1 hr.

Animal # 494

Site	Treatment
A	5 µL of 10% L in $CHCl_3$
B	25 µL of Charcoal waste stream
C	5 µL of 10% HD in $CHCl_3$
D	25 µL of Red waste stream
E	5 µL of 10% HN in $CHCl_3$
F	25 µL of Blue waste stream
G	1 µL of neat HD
H	

Animal # 496

Site	Treatment
A	5 µL of 10% HD in $CHCl_3$
B	25 µL of Red waste stream
C	5 µL of 10% HN in $CHCl_3$
D	25 µL of Blue waste stream
E	5 µL of 10% L in $CHCl_3$
F	25 µL of Charcoal waste stream
G	1 µL of neat HD
H	

Animal # 497

Site	Treatment
A	5 µL of 10% HN in $CHCl_3$
B	25 µL of Blue waste stream
C	5 µL of 10% L in $CHCl_3$
D	25 µL of Charcoal waste stream
E	5 µL of 10% HD in $CHCl_3$
F	25 µL of Red waste stream
G	1 µL of neat HD
H	

Animal # 499

Site	Treatment
A	5 µL of 10% HN in $CHCl_3$
B	25 µL of Blue waste stream
C	5 µL of 10% L in $CHCl_3$
D	25 µL of Charcoal waste stream
E	5 µL of 10% HD in $CHCl_3$
F	25 µL of Red waste stream
G	1 µL of neat HD
H	

Animal # 494	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		4	0	1	0	3	2	3
Epidermal Necrosis		4	0	4**	0	4	2	4***
Follicular Necrosis		4	0	4	0	4	0	4
Dermal Necrosis		0*	0	3	0	0	0	2
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		3	0	0	0	0	0	0
Pustular Epidermitis		0	0	1	0	1	0	0

Animal # 496	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		0	0	0	4	1	0	2
Epidermal Necrosis		4*	0	4*	3	4*	1	4*
Follicular Necrosis		4	0	4	0	4	0	4
Dermal Necrosis		3	0	3	0	4	0	3**
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: *marked ulcer precludes potential blister **mild dermal edema		mod dermal inflam	min dermal inflam	mod dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam

Animal # 497	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		1	2	4	0	2	0	2
Epidermal Necrosis		4*	4	4	1***	4*	0	4*
Follicular Necrosis		4	0	4	0	4	0	4
Dermal Necrosis		3	0	0**	0	2	0	2**
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0
Notes: *marked ulceration **moderate dermal edema ***mild epithelial cell edema		mod dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam	mild dermal inflam	mod dermal inflam

Animal # 499	Site	A	B	C	D	E	F	G
Histopathology Markers:								
Microblister		4	2	3	0	4	0	3
Epidermal Necrosis		4	3	4	2	4	0	4
Follicular Necrosis		4	0	4	0	4	0	4
Dermal Necrosis		0	0	2*	0	2	0	1*
Vascular Necrosis		0	0	0	0	0	0	0
Hemorrhage		0	0	1	0	0	0	0
Pustular Epidermitis		1	0	0	0	1	0	0
Note: *mild dermal edema		mod dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	mild dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale 3/25/96
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe Allen W. Singer, DVM

Task 95-38, Phase 3, Day 3

"Fresh" Blue and Red waste streams received 6/19/1996

Key for HGPs #339, 341, 342, and 346 dosed 6/20/1996. Exposure duration - 1 hr.

Animal # 339

Site	Treatment
A	5 μL of 10% HN in $CHCl_3$
B	25 μL of Red waste stream
C	5 μL of 10% L in $CHCl_3$
D	25 μL of Blue waste stream
E	5 μL of 10% HD in $CHCl_3$
F	25 μL of Red waste stream
G	1 μL of neat HD
H	25 μL of Blue waste stream

Animal # 341

Site	Treatment
A	5 μL of 10% HD in $CHCl_3$
B	25 μL of Blue waste stream
C	5 μL of 10% HN in $CHCl_3$
D	25 μL of Red waste stream
E	5 μL of 10% L in $CHCl_3$
F	25 μL of Blue waste stream
G	1 μL of neat HD
H	25 μL of Red waste stream

Animal # 342

Site	Treatment
A	5 μL of 10% L in $CHCl_3$
B	25 μL of Blue waste stream
C	5 μL of 10% HD in $CHCl_3$
D	25 μL of Red waste stream
E	5 μL of 10% HN in $CHCl_3$
F	25 μL of Blue waste stream
G	1 μL of neat HD
H	25 μL of Red waste stream

Animal # 346

Site	Treatment
A	5 μL of 10% L in $CHCl_3$
B	25 μL of Red waste stream
C	5 μL of 10% HD in $CHCl_3$
D	25 μL of Blue waste stream
E	5 μL of 10% HN in $CHCl_3$
F	25 μL of Red waste stream
G	1 μL of neat HD
H	25 μL of Blue waste stream

Animal # 339	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		3	0	4	3	3	0	2	2
Epidermal Necrosis		4	0	4	4	4**	0	4	2
Follicular Necrosis		4	0	4	0	4	0	4	0
Dermal Necrosis		0	0	2*	0	2	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0	0
Pustular Epidermitis		1	0	1	1	1	0	0	0
Notes: *moderate dermal edema **focal ulceration(s)		mod dermal inflam	min dermal inflam	mod dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam	mild dermal inflam	min dermal inflam

Animal # 341	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		2	2	2	0	3	0	2	0
Epidermal Necrosis		4*	4	4*	0	4*	4*	4*	0
Follicular Necrosis		4	1	4	0	4	2	2	0
Dermal Necrosis		3	1	2	0	3**	3	3**	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		1	0	0	0	1	0	1	0
Pustular Epidermitis		0	0	0	0	0	0	0	0
Notes: *focal ulceration(s); **moderate dermal edema		mild dermal inflam	mild dermal inflam	mild dermal inflam	min dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam	min dermal inflam

Animal # 342	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		3	1	3	0	4	3	3	1
Epidermal Necrosis		4	4	4	0	4	4	4	4
Follicular Necrosis		4	0	4	0	4	1	4	4
Dermal Necrosis		0*	0	0*	0	0	0	0	0*
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0	0
Pustular Epidermitis		0	0	1	0	0	0	0	0
Notes: *mild to moderate dermal edema		mild dermal inflam	min dermal inflam	mild dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	mild dermal inflam	min dermal inflam

Animal # 346	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		2	0	2	1	4	0	2	1
Epidermal Necrosis		4	0	4	4	4	0	4	4**
Follicular Necrosis		4	0	4	1	4	0	4	0
Dermal Necrosis		0*	0	0	0	2	0	0*	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	0	0	0	0	0	0
Pustular Epidermitis		0	0	0	0	0	0	0	0
Notes: *moderate dermal edema; **most of surface epithelium artifactually stripped away		mild dermal inflam	min dermal inflam	mod dermal inflam	mild dermal inflam	mild dermal inflam		mild dermal inflam	min dermal inflam

Note: Normal (unaffected) skin presented laterally on all skin sections with lesions.
Histopathological Markers — 6/25/96
Degree of Severity Grading Scale — Allen W. Singer, DVM
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

Task 95-38, Phase 3, Day 4

"Fresh" Blue and Red waste streams received 6/19/1996

Key for HGPs #340, 345, 351, and 352 dosed 6/26/1996. Exposure duration - 1 hr.

Animal # 340

Site	Treatment
A	5 μL of 10% HD in $CHCl_3$
B	25 μL of Blue waste stream
C	5 μL of 10% HN in $CHCl_3$
D	25 μL of Red waste stream
E	5 μL of 10% L in $CHCl_3$
F	25 μL of Blue waste stream
G	1 μL of neat HD
H	25 μL of Red waste stream

Animal # 345

Site	Treatement
A	5 μL of 10% L in $CHCl_3$
B	25 μL of Red waste stream
C	5 μL of 10% HD in $CHCl_3$
D	25 μL of Blue waste stream
E	5 μL of 10% HN in $CHCl_3$
F	25 μL of Red waste stream
G	1 μL of neat HD
H	25 μL of Blue waste stream

Animal # 351

Site	Treatment
A	5 μL of 10% HN in $CHCl_3$
B	25 μL of Blue waste stream
C	5 μL of 10% L in $CHCl_3$
D	25 μL of Red waste stream
E	5 μL of 10% HD in $CHCl_3$
F	25 μL of Blue waste stream
G	1 μL of neat HD
H	25 μL of Red waste stream

Animal # 352

Site	Treatment
A	5 μL of 10% HD in $CHCl_3$
B	25 μL of Red waste stream
C	5 μL of 10% HN in $CHCl_3$
D	25 μL of Blue waste stream
E	5 μL of 10% L in $CHCl_3$
F	25 μL of Red waste stream
G	1 μL of neat HD
H	25 μL of Blue waste stream

Animal # 340	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		2*	3	3	0	3	3	0	0
Epidermal Necrosis		4**	4	4	0	4	4	4	1
Follicular Necrosis		4	2	4	0	4	0	4	0
Dermalal Necrosis		2	0	1	0	0***	0	3***	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		2	0	0	0	2	0	2	0
Pustular Epidermitis		0	0	0	0	0	0	0	0
Notes: *at edge of ulcer **mild ulceration ***mild dermal edema		mod dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	min dermal inflam	min dermal inflam

Animal # 345	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		3*	0	2	1	1	0	1	2
Epidermal Necrosis		4**	0	4	4	4**	0	4	4
Follicular Necrosis		3	0	4	1	4	0	4	1
Dermal Necrosis		3	0	0	0	3	0	2***	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		2	0	1	0	2	0	1	0
Pustular Epidermitis		0	1	0	0	0	0	0	0
Notes: *at one edge of ulcer **marked ulceration present ***mild dermal edema		mod dermal inflam	min dermal inflam	mild dermal inflam	mild dermal inflam	mod dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam

Animal # 351	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		1	2	4	0	1*	2	1	0
Epidermal Necrosis		4	4	4	0	4**	4	4	0
Follicular Necrosis		4	1	3	0	4	1	4	0
Dermal Necrosis		0	0	0	0	3	0	3	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		0	0	1	0	1	0	1	0
Pustular Epidermitis		0	0	0	0	0	0	0	0
Notes: *at one edge of ulcer **marked ulceration present		mild dermal inflam	min dermal inflam	mod dermal inflam		mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam

Animal # 352	Site	A	B	C	D	E	F	G	H
Histopathology Markers:									
Microblister		1*	0	2	1	3	0	2	2
Epidermal Necrosis		4**	0	4**	4	4	0	4**	4
Follicular Necrosis		4	0	3	0	4	0	4	1
Dermal Necrosis		2	0	1	0	0***	0	3	0
Vascular Necrosis		0	0	0	0	0	0	0	0
Hemorrhage		2	0	1	0	1	0	1	0
Pustular Epidermitis		0	0	1	1	0	0	0	0
Notes: *at edge of ulcer **moderate ulceration ***mild dermal edema		mod dermal inflam	min dermal inflam	mod dermal inflam	mod dermal inflam	mild dermal inflam		mod dermal inflam	mild dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

7/1/96
Allen W. Singer, DVM

Task 95-38, Phase 3, Day 5

Blue and Red waste streams received 11/28/1995; Charcoal waste stream received 1/25/96.

Equal volumes of waste streams and 10% HD, HN and L solutions - 10 μL

Key for HGPs #383, 385, 389, and 400 dosed 8/13/1996. Exposure duration - 1 hr.

Animal # 383

Site	Treatment
A	10 μL of 10% L in $CHCl_3$
B	10 μL of Blue waste stream
C	10 μL of 10% HD in $CHCl_3$
D	10 μL of Charcoal waste stream
E	10 μL of 10% HN in $CHCl_3$
F	10 μL of Red waste stream

Animal # 385

Site	Treatment
A	10 μL of 10% HN in $CHCl_3$
B	10 μL of Red waste stream
C	10 μL of 10% L in $CHCl_3$
D	10 μL of Blue waste stream
E	10 μL of 10% HD in $CHCl_3$
F	10 μL of Charcoal waste stream

Animal # 389

Site	Treatment
A	10 μL of 10% HN in $CHCl_3$
B	10 μL of Red waste stream
C	10 μL of 10% L in $CHCl_3$
D	10 μL of Blue waste stream
E	10 μL of 10% HD in $CHCl_3$
F	10 μL of Charcoal waste stream

Animal # 400

Site	Treatment
A	10 μL of 10% HD in $CHCl_3$
B	10 μL of Charcoal waste stream
C	10 μL of 10% HN in $CHCl_3$
D	10 μL of Red waste stream
E	10 μL of 10% L in $CHCl_3$
F	10 μL of Blue waste stream

MREF Task 95-38
G1555-38A

Animal # 383	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		3	0	2	0	3	0
Epidermal Necrosis		4	0	4	0	4	0
Follicular Necrosis		4	0	4	0	3	0
Dermal Necrosis		0*	0	0	0	0	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		2	0	1	0	0	0
Pustular Epidermitis		0	1	0	0	1	0
Notes: *moderate dermal edema		mod dermal inflam		mild dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam

Animal # 385	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		4	0	4	1	3	0
Epidermal Necrosis		4	0	4	1**	4	0
Follicular Necrosis		4	0	4	0	4	0
Dermal Necrosis		1	0	0*	0	0*	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		0	0	2	0	0	0
Pustular Epidermitis		1	0	0	0	0	0
Notes: *mod dermal edema **vacuolar degeneration of epith cells leading to intra- and subepithelial microblister		marked dermal inflam	min dermal inflam	mod dermal inflam		mild dermal inflam	min dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

8/19/96
Allen W. Singer, DVM

E-33
MREF Task 95-38
61555-38a

Animal # 389	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		3	0	2	2	2	0
Epidermal Necrosis		4	0	4	2	4	1
Follicular Necrosis		2	0	4	1	4	0
Dermal Necrosis		0	0	0*	0	0*	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		1	0	3	0	2	0
Pustular Epidermitis		1	0	0	0	0	1
Notes: *severe dermal edema		mod dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam	mild dermal inflam

Animal # 400	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		3	0	4	0	3	3
Epidermal Necrosis		4	0	4	0	4	2
Follicular Necrosis		4	0	2	0	4	1
Dermal Necrosis		0*	0	0*	0	0**	0
Vascular Necrosis		0	0	0	0	1	0
Hemorrhage		0	0	1	0	3	1
Pustular Epidermitis		1	0	1	0	0	0
Notes: *mild dermal edema **severe dermal edema		mod dermal inflam		mod dermal inflam	min dermal inflam	mod dermal inflam	mild dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe

8/19/96
Allen W. Singer, DVM

Task 95-38, Phase 3, Day 6

"Fresh" Charcoal waste stream received 8/29/96.

25 μL of freshly prepared Charcoal waste stream and 5 μL of 10% HD, HN and L solutions

Key for HGPs #379, 380, 387, and 388 dosed 8/29/1996. Exposure duration - 1 hr.

Animal # 379

Site	Treatment
A	5 μL of 10% L in $CHCl_3$
B	25 μL of Charcoal waste stream
C	5 μL of 10% HD in $CHCl_3$
D	25 μL of Charcoal waste stream
E	5 μL of 10% HN in $CHCl_3$
F	25 μL of Charcoal waste stream

Animal # 380

Site	Treatment
A	5 μL of 10% HN in $CHCl_3$
B	25 μL of Charcoal waste stream
C	5 μL of 10% L in $CHCl_3$
D	25 μL of Charcoal waste stream
E	5 μL of 10% HD in $CHCl_3$
F	25 μL of Charcoal waste stream

Animal # 387

Site	Treatment
A	5 μL of 10% HD in $CHCl_3$
B	25 μL of Charcoal waste stream
C	5 μL of 10% HN in $CHCl_3$
D	25 μL of Charcoal waste stream
E	5 μL of 10% L in $CHCl_3$
F	25 μL of Charcoal waste stream

Animal # 388

Site	Treatment
A	5 μL of 10% L in $CHCl_3$
B	25 μL of Charcoal waste stream
C	5 μL of 10% HD in $CHCl_3$
D	25 μL of Charcoal waste stream
E	5 μL of 10% HN in $CHCl_3$
F	25 μL of Charcoal waste stream

MREF Task 95-38
G1555-38A

Animal # 379	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		4	0	2	0	3	0
Epidermal Necrosis		4	1**	4	1**	4	1**
Follicular Necrosis		4	1**	4	1**	4	1**
Dermal Necrosis		0	0	0*	0	0	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		2	0	2	0	1	0
Pustular Epidermitis		0	0	0	0	0	0
Notes: *moderate dermal edema; **random single-cell necrosis noted		mod dermal inflam	min dermal inflam	mild dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam

Animal # 380	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		4	0	4	0	3	0
Epidermal Necrosis		4	1*	4	1*	4	0
Follicular Necrosis		4	1*	4	1*	4	1*
Dermal Necrosis		1	0	2**	0	3**	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		0	0	1	0	0	0
Pustular Epidermitis		0	0	0	0	0	0
Notes: *random single cell necrosis **mod dermal edema; focal ulcer in area of necrosis		mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam	mod dermal inflam	min dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.
Histopathological Markers: Degree of Severity Grading Scale 9/9/96
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe Allen W. Singer, DVM

Animal # 387	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		3	0	2	0	4	0
Epidermal Necrosis		4	1**	4	1**	4	1**
Follicular Necrosis		4	1**	3	1**	4	1**
Dermal Necrosis		0	0	0	0	0*	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		0	0	0	0	2	0
Pustular Epidermitis		0	0	0	0	0	1
Notes: *moderate dermal edema; **random single-cell necrosis noted		mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam	mod dermal inflam

Animal # 388	Site	A	B	C	D	E	F
Histopathology Markers:							
Microblister		4	0	4	0	3	0
Epidermal Necrosis		4	1*	4	1*	4	1*
Follicular Necrosis		4	1*	4	1*	2	1*
Dermal Necrosis		0**	0	0**	0	0	0
Vascular Necrosis		0	0	0	0	0	0
Hemorrhage		3	0	1	0	0	0
Pustular Epidermitis		0	0	0	0	1	0
Notes: *random single cell necrosis **mod dermal edema		mild dermal inflam	min dermal inflam	mild dermal inflam	mild dermal inflam	mod dermal inflam	min dermal inflam

Note: Some normal (unaffected) skin present at one or both ends of each section where lesions were present.

Histopathological Markers: Degree of Severity Grading Scale 9/9/96
0 = Normal; 1 = Minimal; 2 = Intermediate; 3 = Moderate; 4 = Severe Allen W. Singer, DVM

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